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Chesapeake Bay Water Quality
Monitoring Program

Nutrient/Bioassay
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Covering the Period
August 1990 - December 2001

May 2002 Report

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Nutrient-Addition Bioassays in Chesapeake Bay

to Assess Resources Limiting Algal Growth

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Executive Summary

In August, 1990 we initiated a research and monitoring program on resource limitation of phytoplankton growth in Chesapeake Bay. The goal of this work is to provide information on the temporal and spatial variation of resource limitation of algal growth rates in order to create a scientific basis for evaluating regional strategies of nutrient management in Chesapeake Bay watersheds. To accomplish this we have employed monthly bioassays at 9-10 stations to measure light and nutrient limitation (resource limitation) of phytoplankton growth in MD waters of Chesapeake Bay and its tributaries.

Responses of phytoplankton growth rates in the bioassays showed considerable variation. 70% of the 925 bioassays conducted between August 1990 and Dec. 2001 responded to either nitrogen (N) or phosphorus (P), indicating nutrient limitation. Nutrient saturation or light limitation (no response to nutrient additions) was less common (15%), except at the tributary tidal fresh stations where nutrient concentrations and turbidity are high. There were large seasonal and spatial variations in resource limitation, and it was not possible to characterize phytoplankton growth at any station as limited predominantly by a single resource over the course of a year.

There was clear evidence for a seasonal progression in the dominance of light, P, and N in controlling algal growth rates during winter, spring, and summer/fall. During winter (Dec.-Feb.), light and phosphorus exerted dominant control over algal growth rates due to the combination of low temperatures, deep mixing, high freshwater discharge, high turbidity, and abundant nutrients. Nutrient-replete or slightly P-limited algal populations were mixing within a surface layer which was too deep and turbid (optically shallow) for significant net growth in situ. During spring (Mar.-May), P was the dominant control over algal growth rates in mesohaline waters of Chesapeake Bay. Dissolved inorganic nitrogen (DIN) was usually abundant because of high freshwater discharge, but the importance of P as a controlling resource declined in late spring at the end of May or early June, coincident with the onset of bottom water anoxia and decline of DIN in the water column. During the summer and early fall (July-Oct.), N exerted dominant control over growth rates because DIN was usually depleted except near freshwater sources. From September into winter the importance of N declined, and P responses increased as DIN was replenished by increasing runoff. By November, P occasionally (1992, 1994, 1999) became the dominant factor in the main Bay controlling growth rates, usually after fall turnover and reaeration of sediments in October. During the late fall, the importance of both P and N in controlling growth rates declined and light increased in importance as a limiting resource as nutrients increased.

Much of this seasonal variation was due to seasonal variations in hydrology. Monthly average N indices were inversely related to river flow, and P and light indices increased with increasing river flow. These relationships are due to the high turbidity and high DIN/PO_4 of incoming river water, which creates conditions conducive to light limitation, followed by P limitation, with excess N, if the water clears sufficiently.

There was also significant spatial variability in these data. The mesohaline Patuxent, in particular, showed little response to P additions compared to other mesohaline stations, although large and consistent N responses were observed each summer. This suggests weak P limitation and an abundance of P, probably due to the large amounts of P-rich wastewater which flows into this tributary compared to other areas. In addition, algal growth at the tidal fresh stations was primarily nutrient-saturated and controlled by light from fall through spring. Summer (high temperature and low flows) was the period of greatest nutrient limitation.

We have made considerable progress in identifying simple diagnostic tools other than bioassays to measure resource limitation. P deficiency is readily identified by alkaline phosphatase activity >10 nmol PO_4 µg $chla^{-1}$ h^{-1}. In addition, C:P and N:P ratios of particulate organic matter in excess of Redfield proportions and DIN/PO_4 >300:1 are also associated with P deficiency and P responses in the bioassays. N responses may be predicted by DIN concentrations <5 µM (0.070 mg NO_3-N ℓ^{-1}). Furthermore, C:P and N:P particulate ratios less than Redfield proportions and DIN/PO_4 <30:1 are also useful indicators of N deficiency and N response in the bioassays. We have also had success with the use of an intracellular amino acid ratio (glutamine/glutamate) as an indicator of N limitation. Our goal with these indicators has been to use field data to map resource limitation over large areas of the Bay where we may not have bioassay information. Such maps of resource limitation may be used as monitoring tools to provide finer resolution of temporal and spatial variations in resource limitation and to examine in a cost-effective manner the effects of watershed management actions on adjacent waters of the Chesapeake Bay. In separate reports to DNR submitted in previous years (e.g., Fisher et al. 1992b, 1999a, 1999b), we presented detailed analyses of the relationships between twenty two indicators and the indices of N, P, and Light limitation, including statistical models which predict resource limitation based on single and multiple indicators. In 2001, with the help of Dr. Elgin Perry, we also made a simple, combined statistical model which predicts N, P, and Light limitation with better accuracy than previous attempts. A separate report on this new development will be submitted separately with Elgin Perry.

In 2000 we initiated a new kind of bioassay in which light as well as nutrients are manipulated ("resource-addition bioassays"). These are designed to examine nutrient-saturation and light limitation in more detail and to help us distinguish between light and nutrient limitation of algal growth and biomass accumulation. Although we have done only a few of these resource-addition bioassays to date, the results suggest that light adaptation during the bioassays and grazing control of algal growth may be important processes which have influenced our results in the past, particularly our classification of light-limited growth (NOR) in winter. This was one of the hardest categories of algal growth response to predict with indicator models, and misclassification of bioassay responses may be one of the reasons. We will report more details on these bioassays in future reports when sufficient data are available.

Using the data summarized here we have estimated interannual trends in resource

limitation of algal growth to evaluate the effects of watershed inputs. The interannual variability in resource limitation appears to be due primarily to interannual changes in hydrology and nutrient inputs. In addition to years with unusual hydrology, there was a broad increase in the current status of N and P limitation (1999-2001) for many stations compared to our initial period of record (1990-1992). The only exceptions were the mesohaline Choptank (ET5.2, no significant change in N status) and the tidal fresh and mesohaline Potomac and tidal fresh Patuxent stations (TF2.3, LE2.3, LE1.1, no significant change in P status). The pattern of increasing N and P limitation at other stations reflects lower nutrient saturation and light limitation and indicates improving water quality conditions. Although the data suggest that water quality in the MD portion of the Bay has generally improved somewhat in the 1990's, it is also clear that additional reductions in nutrient inputs from surrounding watersheds are still required in order to achieve the water quality goals of the Bay Program.

Introduction

There is considerable variability in the nutrient(s) limiting algal growth and accumulation in aquatic systems. Nitrogen (N), phosphorus (P), and silicon (Si) are all candidate limiting elements (Caraco et al. 1987, Caraco 1988, Conley and Malone 1992, Elser et al. 1990, Fisher et al. 1992, Hecky and Kilham 1988, Howarth 1988, Smith et al. 1989, Webb 1988). Temperate lakes appear to be primarily P-limited (e.g., Dillon and Rigler 1974, Schindler 1977) because of high N:P loading, N fixation, and sediment retention of P (Levine and Schindler 1992). However, there is recognition of the importance of N in temperate lakes (e.g., Elser et al. 1990), some of which appear to become N-depleted under summer stratification (Dodds and Priscu 1990) and with increasing eutrophication (McCauley et al. 1989). Marine waters are often considered N-limited (Ryther and Dunstan 1971, Howarth 1988) due to enhanced sediment release of P relative to N (Nixon et al. 1980, Caraco et al 1990), slow N fixation (Howarth et al. 1988), and high denitrification (Seitzinger 1988), although iron (Fe) may limit algal growth in open ocean areas far from land (e.g., Martin et al. 1994). The major difference between primarily N-limited marine systems and primarily P-limited freshwater systems appears to be the greater capacity of marine sediment to release P (Caraco et al. 1990) due to seasonal displacement of $H_2PO_4^-$ by HS^- within Fe oxyhydroxide complexes (Krom and Berner 1981). In addition to N and P, Si may also limit silicious phytoplankton such as diatoms in lakes (Tilman 1982), estuaries (Conley and Malone 1992), and coastal regions (Malone et al. 1980, Officer and Ryther 1980).

Estuaries are the transition from freshwater to true marine systems. In estuaries and coastal ponds, there is evidence for seasonal and spatial variations in the limiting nutrient (Caraco et al. 1987, Caraco 1988, D'Elia et al. 1986, Fisher et al. 1992a, 1999, Webb 1988). For example, in the Patuxent and York subestuaries of Chesapeake Bay, meso-scale bioassays indicated P limitation of biomass accumulation during winter and spring and N limitation during summer (D'Elia et al. 1986, Webb 1988). Fisher et al. (1992a, 1999) and Pennock and Sharp (1994) present evidence for similar seasonal alternations in light, P, and N limitation of algal growth rates in the Chesapeake and Delaware estuaries. There are also reports of P limitation in estuaries and coastal areas influenced by large rivers (D'Elia et al. 1986, Harrison et al. 1990, Fisher et al. 1992, Pennock and Sharp 1994).

An understanding of the variability of the controls on algal growth is important. In addition to contributing to our basic knowledge of the structure and function of aquatic systems, studies of nutrient limitation have direct application to water quality management. Large sums of money will be spent for future nutrient reduction strategies in watersheds of estuaries such as Chesapeake Bay. Wastewater treatment plants may be upgraded, land uses may be limited, and best management practices may be mandated for the agricultural community. The cost and type of nutrient reduction strategy varies considerably with the nutrient which is targeted (e.g., Clasen and Bernhardt 1987, Wetzel 1983), and a sound scientific foundation is needed for management decisions concerning nutrient reductions.

This is a progress report summarizing data from August 1990 - December 2001. Although we present selected data covering the entire project period of August 1990 to December 2001, a previous report (Fisher et al. 1992b) summarized the details of the data from August 1990 to June 1992. In July 1992 we changed our protocols for nutrient addition bioassays, and we added two new indicators of P and N deficiency: alkaline phosphatase activity and the ammonium enhancement ratio. In July 1994 we also added intracellular ratios of amino acids as an indicator of N deficiency. This report gives cumulative statistics on bioassays from August 1990 to December 2001; however, in the body of the report we emphasize data gathered from July 1992 to December 2001.

In this report we use the term "resource limitation" to denote control of algal growth by both light and nutrients. Although nutrients such as N, P, Si, etc. are well known to control algal populations in lakes, estuaries, and oceans (references cited above), availability of light in the water column is essential for algal photosynthesis. However, in many estuaries the turbidity restricts the amount of light available, and light may be the limiting resource in many turbid, nutrient-rich areas (e.g., Cloern 1987). We therefore prefer the more general term "resource limitation," and we routinely examine light and nutrients as potential controls on algal growth.

Methods

Bioassays are sensitive measures of nutrient limitation of algal growth rates (Elser et al. 1988). Nutrient limitation is assessed by changes in growth rates over several days after additions of the limiting nutrient alone and in combination with other nutrients. In the bioassays reported here on water samples from MD waters of the Chesapeake, changes in phytoplankton growth rates were estimated as changes in algal biomass (chlorophyll a, µg chla ℓ^{-1}) and in photosynthetic potential (^{14}C-CO_2 incorporation at saturating light, 1 hour at 200 µE m^{-2} s^{-1}). All responses to nutrient additions were normalized to that of a control with no added nutrients. Details of the conceptual basis for the interpretation of the bioassays are presented in Fisher and Gustafson (1994) and Fisher et al. (1999).

The responses in the bioassays appear to be those of the original algal populations in each sample. During 1990-1995, subsamples of each treatment were preserved with buffered Lugol's solution for enumeration of species, although only a subset of 10 bioassays were taxonomically evaluated in detail (Fisher et al. 1992b). The data showed that no exotic species bloomed under the bioassay conditions, and that the bioassay responses were due to increases in the natural populations.

Water samples for the bioassays were subsamples of those obtained during the DNR sampling periods (Table 1). Samples were stored at reduced light and ambient temperature

Table 1. Station descriptions and sampling schedule for bioassays of resource limitation of phytoplankton growth. The number of samples at each station varies due to the sampling schedule (e.g., tidal fresh stations are sampled only 3 times per year), bad weather, boat mishaps, etc., and numbers of bioassays are cumulative since August, 1990. Turkey Point is a new station at which we initiated sampling in 2000, and we discontinued sampling in Baltimore Harbor in 1993. Abbreviations: tidal fr. = tidal fresh.

sampling area	description	MDE sta.ID	sampling day	our ID#	# of Samples to date
Main Bay stas.	Turkey Pt	CB 2.1	wed.	11	5
	Bay bridge	CB 3.3C	tues.	4	116
	R64 buoy	CB 4.3C	tues.	5	123
	Point-no-point	CB 5.2	mon.	1	126
Baltimore Harbor	Baltimore H.	WT 5.1	NA	8	26
Patux. (tidal fr.)	Nottingham	TF1.5	thurs.	9	55
(mesohaline)	Jack Bay	LE1.1	thurs.	10	122
Potomac (tidal fr.)	Indian Head	TF2.3	mon.	2	57
(mesohaline)	Ragged Pt	LE2.2	mon.	3	123
Choptank (tidal fr.)	Ganey Wharf	ET5.1	tue.	6	54
(mesohaline)	Cambridge	ET5.2	tue.	7	118
Total number	Stations:	11		Bioassays:	925

on the ship and transported on the day of collection to Horn Point Lab for overnight storage in a incubator regulated at ambient water temperature and the photoperiod of the natural light cycle with fluorescent and incandescent light (200 µE m^{-2} s^{-1}). The next day subsamples were taken for initial analyses, and each large volume (~ 20 ℓ) water sample was then subdivided into 3 ℓ subsamples in plastic, transparent cubetainers. See Fig. 1 for a summary.

We perform bioassays of algal growth using additions of inorganic N and P substrates at fixed light levels (% of ambient surface PAR). Incubations are performed at ambient temperature and light. These bioassays use protocols developed by research efforts from 1990 to present as part of the Chespeake Bay monitoring effort funded by MD DNR, VA DEQ and EPA Bay Program. Protocols are versions of those reported by Fisher et al. (1992), Haas and Wetzel (1993), Fisher and Gustafson (1998), and Fisher et al. (1999).

Two major types of bioassays were performed: (1) nutrient-addition bioassays at a single light level (58% of ambient light), and (2) resource-addition bioassays at multiple light levels

Table 2. Nutrient-addition and resource-addition bioassays used to assess resource limitation in Chesapeake Bay. Nutrient addition bioassays are done at only one light level (58 %), with varying nutrient additions. Resource-addition bioassays are done at varying light levels as well as with varying nutrient additions, along with a time series in the 58 % light incubation (current year) and in the 11 % light incubation (coming year). In each type of bioassay, the original water sample is processed for initial conditions and subdivided into treatments with varying nutrient and light levels (resource-limitation bioassays only). The numbers below represent the number of subsamples processed at each light level and nutrient treatment.

Nutrient-addition bioassay:

	% light	control	+N	+P	+NP	total
	100	0	0	0	0	0
	58	2	1	1	1	5
initial sample	34	0	0	0	0	0
	20	0	0	0	0	0
	11	0	0	0	0	0
1	totals	2	1	1	1	6

Resource-addition bioassay:

	% light	control	+N	+P	+NP	total	
	100	1	1	1	1	4	(2000 only)
	58	8	4	4	4	20	(time series)
initial sample	34	1	1	1	1	4	
	20	1	1	1	1	4	
	11	4	4	4	4	16	(time series)
	6	1	1	1	1	4	(added in 2001)
1	totals	16	12	12	12	53	

(see Table 2). The latter were initiated in July 2000 and include a nutrient-addition bioassay, with additional light treatments and a time series at 58% light. We designed the resource-addition bioassay to provide more details on light limitation and to help distinguish light and nutrient limitation.

At the start of each nutrient-addition bioassay, 3 L aliquots of each sample were placed in plastic (LDPE), transparent, cubic containers ("cubetainers"). There were two controls with no additions, a single +N addition (+30 µM NH_4 = 0.42 mg NH_4-N/L), a single +P addition (+2 µM PO_4 = 0.062 mg PO_4-P/L), and a single +N+P addition. The amounts of the nutrient additions were chosen to saturate uptake rates and provide temporary relief from nutrient limitation in order to stimulate algal growth. In addition, we added 30 µM SiO_4 (= 0.84 mg SiO_4-Si/L) to all treatments and controls to eliminate the possibility of Si limitation, although we never obtained a significant Si response when we used a +Si treatment during 1990-1992. Additional treatments occasionally employed are additions of rain water and sewage to test the effect of atmospheric N deposition and sewage on algal populations. Controls and treatments were incubated in shallow (<0.5 m), running water incubators on the HPL pier in the Choptank river at ambient temperature and light attenuated with a 58% transmittance, neutral density screen to reduce photosynthetically active radiation (PAR). Incubations were terminated when the cumulative PAR under the 58% transmittance screen was equivalent to that of an average day for each month (13-50 E m^{-2} d^{-1}, see Fisher et al. 1999). The target PAR value was adjusted monthly based on a 9 y record. Incubations typically last 2-5 d, long enough to permit at least two doublings at maximum growth rates (Eppley 1972). Allowing sufficient time for two doublings provides the potential for a 400% response to nutrient additions relative to controls. The variable time approach compensates for cloudy days or low temperature. Incubations of several days also eliminate the possibility of short term (1-6 h) energy competition between C fixation and nutrient assimilation (Healey 1979; Lean and Pick 1981).

The resource-addition bioassays add additional light treatments to the nutrient-addition bioassay (see Table 2). Layers of screens provide increasing attenuation of ambient surface light down to 11%. In addition, a time series of nutrients and chlorophyll a in the 58% light treatment is added to the initial and final measurements of chlorophyll a and ^{14}C uptake to show the time course of the responses to the manipulated resources. Light is not added as nutrients are in the nutrient-addition bioassays, but light is manipulated as a resource available to phytoplankton populations.

Responses to nutrient additions in treatments were normalized to those of the control without nutrient additions. Responses equivalent to those of the control therefore equal 100 %. Increases in chlorophyll a and carbon fixation rate in a treatment (relative to the control) were usually proportional to each other and were interpreted as indicative of a growth rate increase in response to the addition.

The significance of a response greater than the control was based on decision rules (Table 3). The rules were formulated from a statistical study of the frequency distribution of

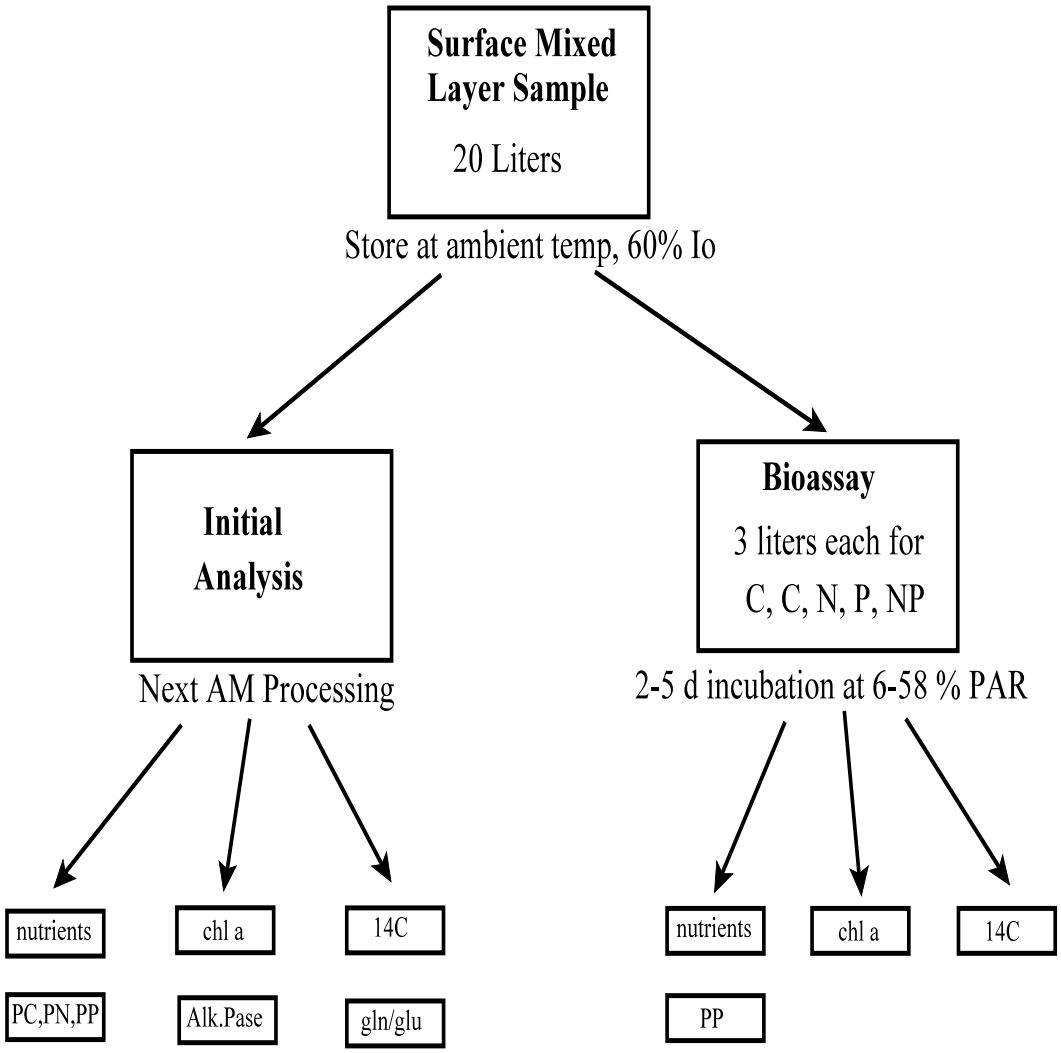

Fig 1. Flow diagram for processing of samples for analysis of nutrient limitation. Abbreviations: I_0 = surface irradiance of PAR (photosynthetically active radiation, 400-700 nm), nutrients = ammonium (NH_4), nitrate (NO_3), phosphate (PO_4), silicate (SiO_4); chl a = chlorophyll a (µg L^{-1}), 14C = ^{14}C-CO_2 uptake (dpm h^{-1}), PC = particulate carbon, PN = particulate nitrogen, PP = particulate phosphorus, Alk.Pase = alkaline phosphatase activity (nmol PO_4 µg $chla^{-1}$ h^{-1}), gln/glu = intracellular glutamine/glutamate ratio.

Table 3. Decision rules used for the classification of responses obtained in nutrient addition bioassays in Chesapeake Bay.

1- Bioassays are classified as "inconsistent" (INC) if two or more observations of chlorophyll a (CHAA) or C fixation (CFIX) in any treatment are < 75% of the control.

2- A treatment is considered significantly greater than the control:

months	if both CHAA and CFIX are >	or if one is >
Dec.-Mar.	120%	140%
Nov., Apr.	130%	160%
May-Oct.	140%	180%

3- Using the treatments significantly greater than the control, bioassays are classified according to the conceptual model in Fig. 2. Classifications with significant responses are "exclusive N" (EXN), "primary N" (PRN), "balanced NP" (BNP), "primary P" (PRP), and "exclusive P" (EXP).

4- Bioassays with no significant responses are classified as "no response" (NOR).

5- Bioassays not conforming to the conceptual model in Fig. 2 are classified as "inconsistent" (INC).

6- Bioassays which are classified as EXN, PRN, or BNP by the above rules, but which also have nitrate concentrations exceeding 10 µM (0.14 mg NO_3-N L^{-1}), are reclassified as "XN1", "XN2", and "XN3", respectively. This is necessary because of the preference of phytoplankton for the experimentally added ammonium over the ambient nitrate already present in the sample. Light-limited samples sometimes respond to the ammonium additions when adequate nitrate is present, giving a false N classification. Since adequate N and P are already present in the sample (NO_3 > 10 µM or 0.14 mg NO_3-N L^{-1} and no response to added PO_4), these reclassified bioassays will be grouped with NOR for statistical summaries. However, they will retain the XN1-XN3 classification in the data files.

the maximum response of each bioassay conducted during the period Aug. 1990 - Dec. 1991 (Vaas and Magnien 1992). Threshholds for a significant response were set at approximately the 90th percentile of the upper tail of the frequency distribution during winter months (Dec. - Mar., 120 % of control), somewhat below the 90th percentile during April and November (130 %), and considerably below the 90th percentile in warm months (May - Oct., 140 %). This approach automatically creates a small bias towards no response in winter months, because small but significant responses may be excluded. However, it was necessary because cumulative experimental errors in bioassays approach 20%. If the November through April threshholds are set at levels lower than 120-140 %, spurious results frequently occurred (e.g., apparent N or P limitation with responses of 105-110 % of control under conditions of low algal biomass in the presence of substantial concentrations of DIN and/or PO_4). The decision rules (Table 3) avoid this problem and provide a more consistent set of bioassay results. The decision rules do not exclude large, significant responses in winter (incubations provide conditions for responses up to 400 %), although these are rarely found. However, application of the decision rules results in a somewhat larger number of bioassays being classified as 'inconsistent' and 'balanced N&P' in comparison with the t tests for significance employed in previous reports by Fisher et al. (1991, 1992).

The results of the bioassays were interpreted using the conceptual model summarized in Fig. 2. Exclusive N or P limitation (EXN or EXP) was considered to occur when additions of the other nutrient had no influence on the chlorophyll a and C fixation responses in the +N+P treatment; i.e., +N or +P was the same as +N+P. Primary N or P limitation (PRN or PRP) occurred when the other nutrient had no significant effect by itself, but significantly elevated the responses of the +N+P over and above that of the primary element alone. Balanced N&P limitation (BNP) occurred when positive responses relative to the control were present only when both were added. No significant responses to any nutrient additions (NOR) were interpreted as nutrient saturation and light limitation. Bioassay results not matching any of the above were classified as 'inconsistent' (INC).

We added three additional classifications to our complete data set in 1997 (see decision rule 6 in Table 2). One of the problems which we have encountered is an apparent response to ammonium additions (+N treatment), when substantial amounts of DIN are present as nitrate. This occasionally occurs at tidal fresh stations with relatively low algal biomass and high DIN. We have decided to interpret these as algal responses to the added ammonium, which is

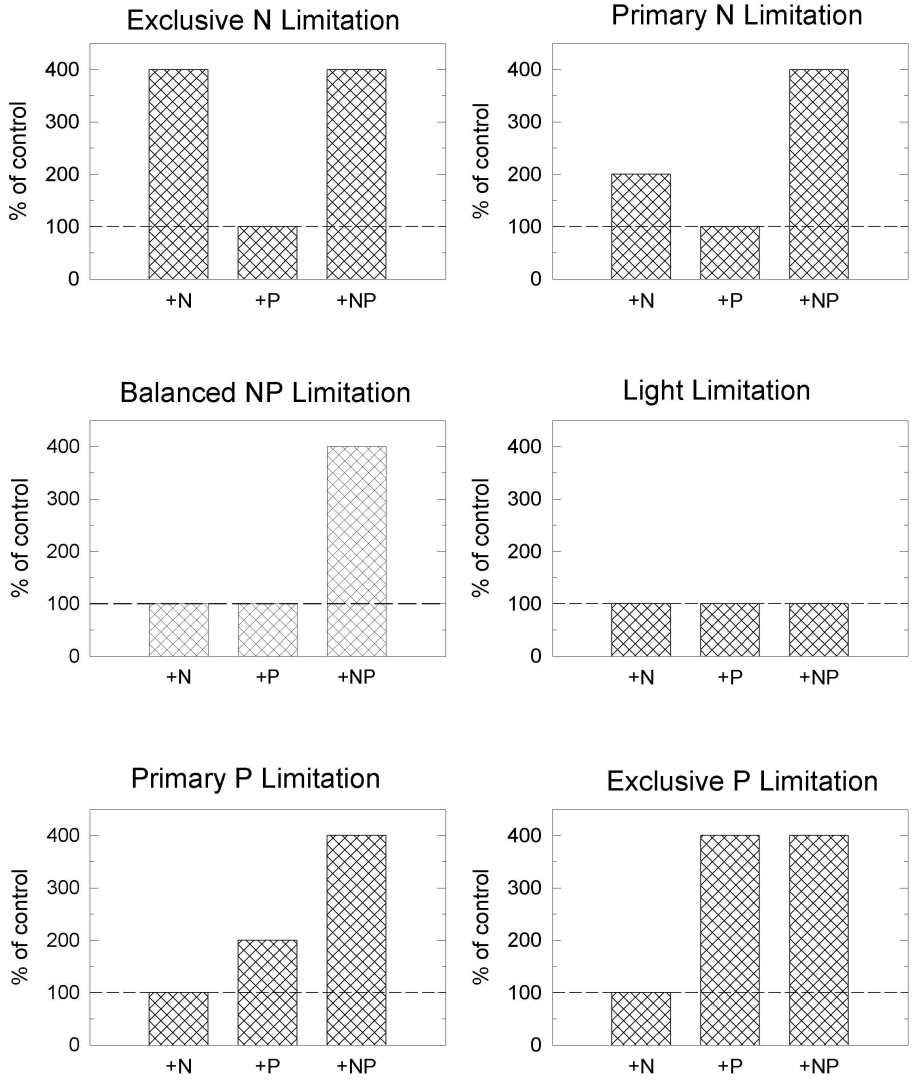

Fig. 2. Conceptual model for the classification of nutrient addition bioassays performed after 1 July 92.

universally used by phytoplankton (McCarthy 1981), in preference to the ambient nitrate under low-growth, light-limited conditions. Therefore, any classifications of EXN, PRN, or BNP with [NO_3] > 10 µM (0.14 mg NO_3-N L^{-1}) were reclassified as XN1, XN2, or XN3, respectively. The 10 µM cutoff was chosen as a level twice the normal range of half-saturation constants for uptake of nitrate, an amount adequate to saturate uptake rates (McCarthy 1981). These new classifications were then grouped with NOR responses in statistical summaries, since it was likely that the populations were really light-limited, with sufficient ambient N (>10 µM NO_3 or 0.14 mg NO_3-N L^{-1}) and P (no response to P additions).

Indices of nutrient and light limitation were computed using the classified bioassays. After excluding the INC bioassays, an index was calculated by weighting each bioassay as shown in Table 4 and dividing by the total number of bioassays. This procedure yields a number ranging from 0, indicating no limitation, to 1, which indicates complete N, P, or light limitation.

Table 4. Weighting factors used to compute indices of N, P, and light limitation of algal growth in Chesapeake Bay using nutrient addition bioassays. Each classified bioassay (see Table 2 and Fig. 2) contributed the amounts shown below to the index, which was then divided by the total number of bioassays. Each index = ($\sum$w)/n, where w is the weighting factor assigned to each of the n bioassays. This results in an index ranging from 0 (no limitation) to 1 (completely limited). Abbreviations: EXN = exclusive N; PRN = primary N; BNP = balanced NP; PRP = primary P; EXP = exclusive P; NOR = no response to added nutrients.

	weighting factors					
Type of Index	EXN	PRN	BNP	PRP	EXP	NOR
N limitation	1.00	0.75	0.50	0.25	0.00	0.00
P limitation	0.00	0.25	0.50	0.75	1.00	0.00
Light limitation	0.00	0.00	0.00	0.00	0.00	1.00

In addition to bioassays, we are using physiological indicators of nutrient limitation. P deficiency is indicated by alkaline phosphatase activity, particulate C:P and N:P, and DIN/PO_4; N deficiency was indicated by intracellular amino acid ratios through Dec. 1998, but this relatively expensive indicator was dropped for financial reasons. [DIN], DIN/PO_4, and particulate N:P continue to be used as indicators of N limitation. Details of the alkaline phosphatase method is described below.

Alkaline phosphatase is a cell surface enzyme, the activity of which is enhanced under P stress (Healey and Hendzel 1979). Duplicate 15 ml subsamples from each station were incubated at *in situ* temperature with 10 µM methy-umbelliferyl phosphate (MUF-PO_4) for 15-30 minutes, depending on season (shorter incubations in spring, longer at other times of year). Hydrolysis of the non-fluorescent MUF-PO_4 by alkaline phosphatase activity in the live planktonic community was indicated by fluorescence of MUF at pH 10. Rates of hydrolysis were normalized to chlorophyll a.

Statistical analyses of the data presented here followed standard conventions. Data

were examined for normality and analyzed with parametric or non-parametric measures, as appropriate. Significance levels were reported as not significant (NS, $p>0.05$), significant (*, $0.05>p>0.01$), and highly significant (**, $0.01>p$). Relationships between variables were evaluated with SigmaStat v.2 (SPSS), and curve fitting was done with SigmaPlot v.6.0 (2000, SPSS). The significance of non-linear relationships was evaluated by a χ^2 test of the increase in r^2 compared to that of a linear fit (Sokal and Rohlf 1995).

More details on this project are available from the authors. In the electronic version, all graphs are provided as jpg files exported from SigmaPlot 2000 v.6. The original Sigmaplot files or data used in those plots are available by email from the authors (email addresses are on the front cover), and a complete copy of this report is posted on the following website:

www.dnr.state.md.us/bay/monitoring/limit/index.html

Results

Spatial and temporal variability of nutrient-addition bioassays

Planktonic populations at all stations showed a variety of responses to added nutrients over the period of study (Table 5). Of the 925 bioassays conducted between August 1990 and December 2001, we classified 651 (70 %) within the five major classes exhibiting significant responses to either N or P. Light limitation (= nutrient saturation or NOR, no response to nutrient additions) was less common (138 or 15 %), although NOR was the dominant response at the tidal fresh stations. Inconsistent bioassays, those not meeting the criteria of Table 3, were a small percentage of the total (64 or 7 %), and bioassays classified as XN1, XN2, and XN3 (high ambient nitrate with a significant response to experimentally added ammonium) were also a small group (72 or 8 %).

Examples of bioassays showing light, primary N, and primary P limitation are shown in Fig. 3. In the example of light limitation, there were responses in all treatments relative to the

Table 5. Summary of bioassay results at the ten stations over the period August, 1990 through Dec., 2001. The number of bioassays classified in each type of response at each station are given. Abbreviations: EXN = exclusive N limitation, PRN = primary N limitation, BNP = balanced NP limitation, PRP = primary P limitation, EXP = exclusive P limitation, NOR = no response (nutrient saturation, light limitation), INC = inconsistent, and XN = N responding bioassays with $NO_3 > 10$ µM (0.14 mg NO_3-N L^{-1}). Within each group, stations are arranged from most fresh to most saline, with the main bay stations first and tributary stations arranged from north to south and from tidal fresh to mesohaline. The last three lines are indices of N, P, and light limitation computed from bioassay responses. Baltimore Harbor is no longer a currently sampled station. CB 2.1 sampling started July 2000.

	main bay stations				Balt.H.	Choptank River		Patuxent River		Potomac River		
type	CB2.1	CB3.3C	CB4.3C	CB5.2	WT5.1	ET5.1	ET5.2	TF1.5	LE1.1	TF2.3	LE2.2	Σ
EXN	0	6	4	2	0	12	15	19	21	0	6	85
PRN	0	19	35	36	0	3	40	4	54	1	44	236
BNP	0	15	17	29	0	1	9	1	16	1	17	106
PRP	2	32	41	25	8	2	21	0	8	10	26	175
EXP	2	7	8	7	4	0	1	2	3	9	6	49
NOR	0	18	8	11	6	18	15	19	11	20	12	138
INC	0	8	4	13	2	6	8	3	7	4	9	64
XN	1	11	6	3	6	12	9	7	2	12	3	72
Σ = 5	116	123	126	26	54	118	55	122	57	123	925	
N index = 0.12	0.37	0.43	0.45	0.11	0.42	0.54	0.50	0.63	0.09	0.49	0.45	
P index = 0.88	0.45	0.50	0.45	0.56	0.08	0.31	0.08	0.27	0.43	0.40	0.37	
Light in.= 0.00	0.18	0.07	0.10	0.33	0.50	0.15	0.42	0.10	0.48	0.11	0.18	

initial conditions (=100%), including the control. Chlorophyll a (white bars) showed the strongest increase. However, there were no significant increases in any treatments relative to the control. We interpret this as a response from an energy-starved algal population deeply mixing in an optically shallow water column (light limitation). The other two examples of primary N and primary P limitation showed nearly equal increases in both chlorophyll a and ^{14}C fixation in the N or P treatments, but the greatest responses occurred in the +N+P treatments. Hence one nutrient supplied the primary limiting element, but the small surplus of the other was depleted when an excess of the primary limiting element became available. Particulate phosphorus (PP) responses occurred only when P was added (+P, +N+P) and were largest under primary P limitation. However, increases in PP were also significant in the primary N bioassay, indicating that internal P pools were not completely filled.

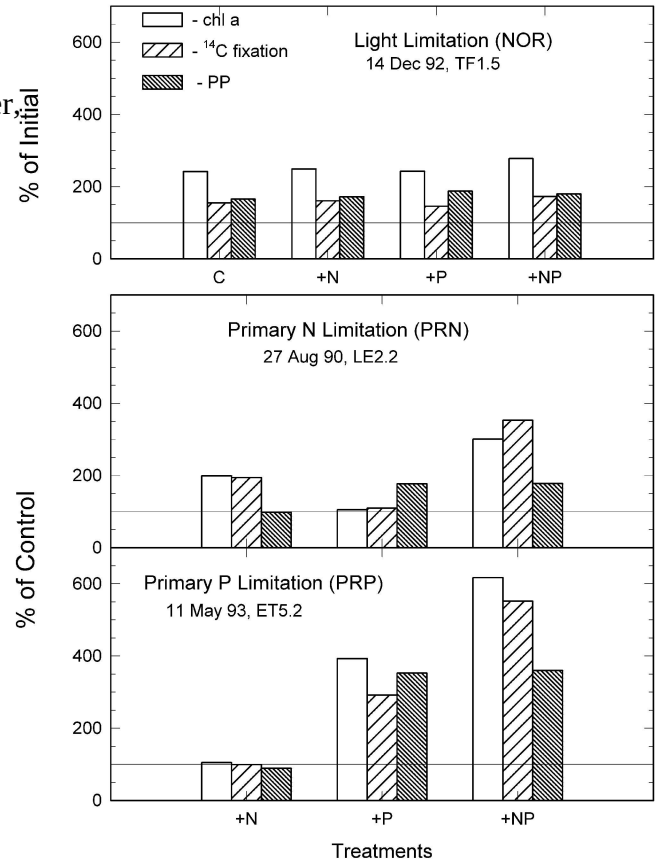

Fig. 3. Examples of bioassays showing light (NOR), primary N (PRN), and primary P (PRP) limitation. C = control, +N = NH_4 addition, +P = PO_4 addition, +NP = NH_4 and PO_4 addition. Horizontal line (100%) = no response relative to control or initial.

There was a broad range of response types observed at the MD stations in Chesapeake Bay. We observed light limitation (NOR), and the most N limited response (EXN) through the most P limited response (EXP). The frequency of each bioassay classification at all stations is shown in Fig. 4. This graph clearly shows that Chesapeake Bay cannot be characterized as limited primarily by either light, N, or P. Most of the rest of this report describes the complex spatial and temporal components of resource limitation in the Bay.

There was considerable spatial variability in the bioassay results. Indices of algal growth rate limitation at each station over the period of study varied between 0.0-0.6 (Table 5), and no station could be categorized as limited by only one resource (light, N, or P). Note that station CB2.1 was newly added in 2000, and only 5 bioassays from spring and summer conditions are available for this station. Until more data are available, we exclude this station below from the analyses, although the available data suggest a tidal fresh station with excess N

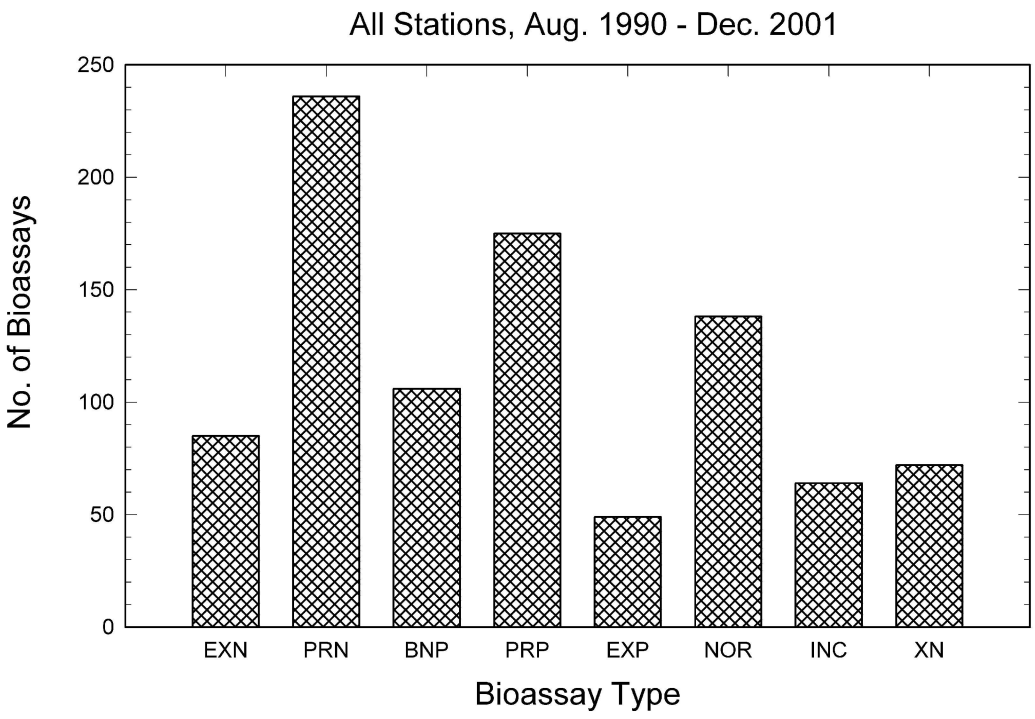

Fig. 4. Frequencies of bioassay response types for all stations over the period Aug. 1990 - Dec. 2001. Total bioassay number = 925.

and P deficiency due to the station's proximity to the Susquehanna River. Based on their responses to nutrient additions, the stations (excluding CB2.1) fell into three major groups:

Group A: the three mesohaline main bay stations (CB3.3C, CB4.3C, and CB5.2) plus the Choptank and Potomac mesohaline stations (ET5.2 and LE2.2)

Group B: the Patuxent mesohaline station (LE1.1)

Group C: the three tidal fresh stations (ET5.1, TF1.5, and TF2.3).

The five mesohaline stations (group A) showed regular seasonal changes in bioassay responses (see Fig. 5 for an example). During winter (December - February), there were usually weak or no significant responses to N or P additions relative to the control (light limitation or nutrient saturation- see Fig. 3). During spring (March - May), there were usually no significant responses to N additions, but algal growth rates responded to P and N+P (primary P limitation - see Fig. 3). June was a transition month during which P responses declined and N responses increased. During summer (July - September), there were usually no

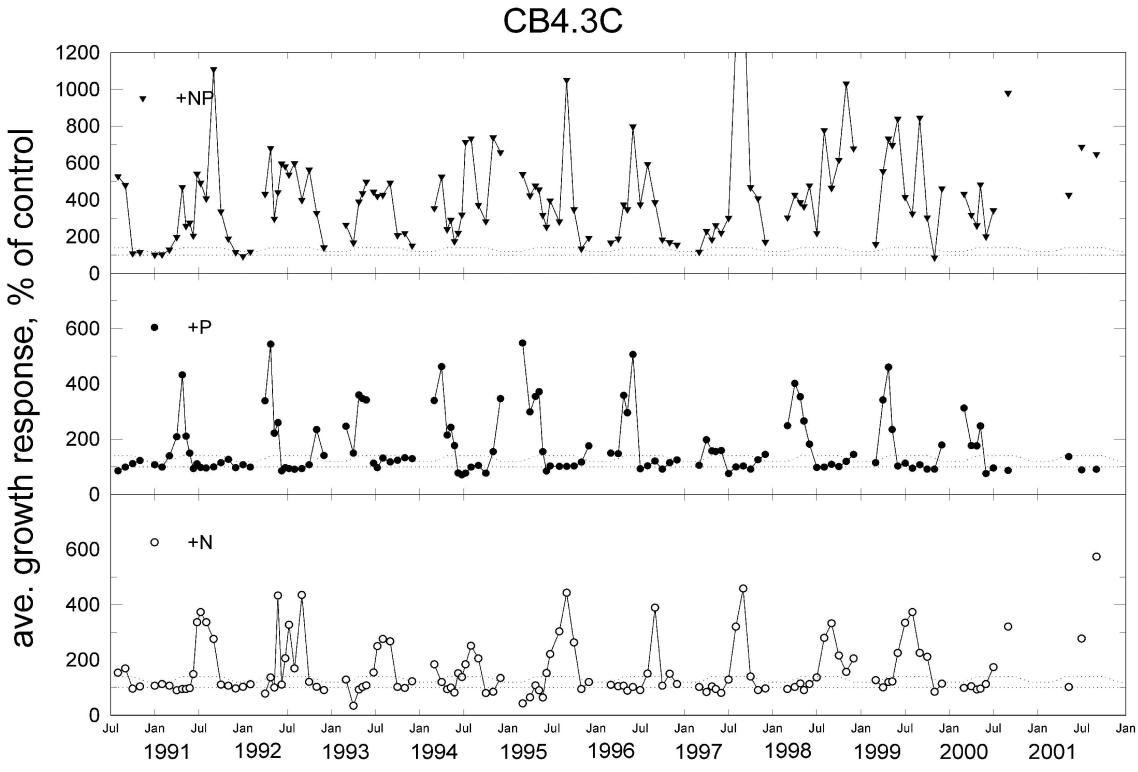

Fig. 5. Bioassay results at the mesohaline main bay station CB4.3C. Straight dotted lines represent 100% of control (no response), and fluctuating dotted lines represent seasonally varying thresholds for significant responses.

responses to P alone, but growth rates responded to additions of N and N+P, which we interpreted as primary N limitation. During fall (October-November), P responses were again sometimes observed before a return to winter light limitation, most notably in 1992 and 1995 (middle panel Fig. 5).

The seasonal progression in the limiting resource for the five similar mesohaline stations can be visualized in Fig. 6 (CB4.3C). The top panel of Fig. 6 illustrates the monthly mean flow of the Susquehanna River, and the lower panels show average monthly indices of resource limitation over the period of record. Light and P are the primary limiting resources in winter (Dec.-Feb.); P is the primary limiting resource during spring (Mar.-May); and, N is the primary limiting resource in summer and early fall (July-Sept.), with a return to P limitation in late fall.

River discharge is a major control on the seasonal changes observed at Group A stations. There are highly significant exponential relationships between the average monthly N and P indices and the average Susquehanna River discharge for each month (Fig. 7). As river discharge increases, the P index exponentially approaches 0.8 and the N index approaches 0.2.

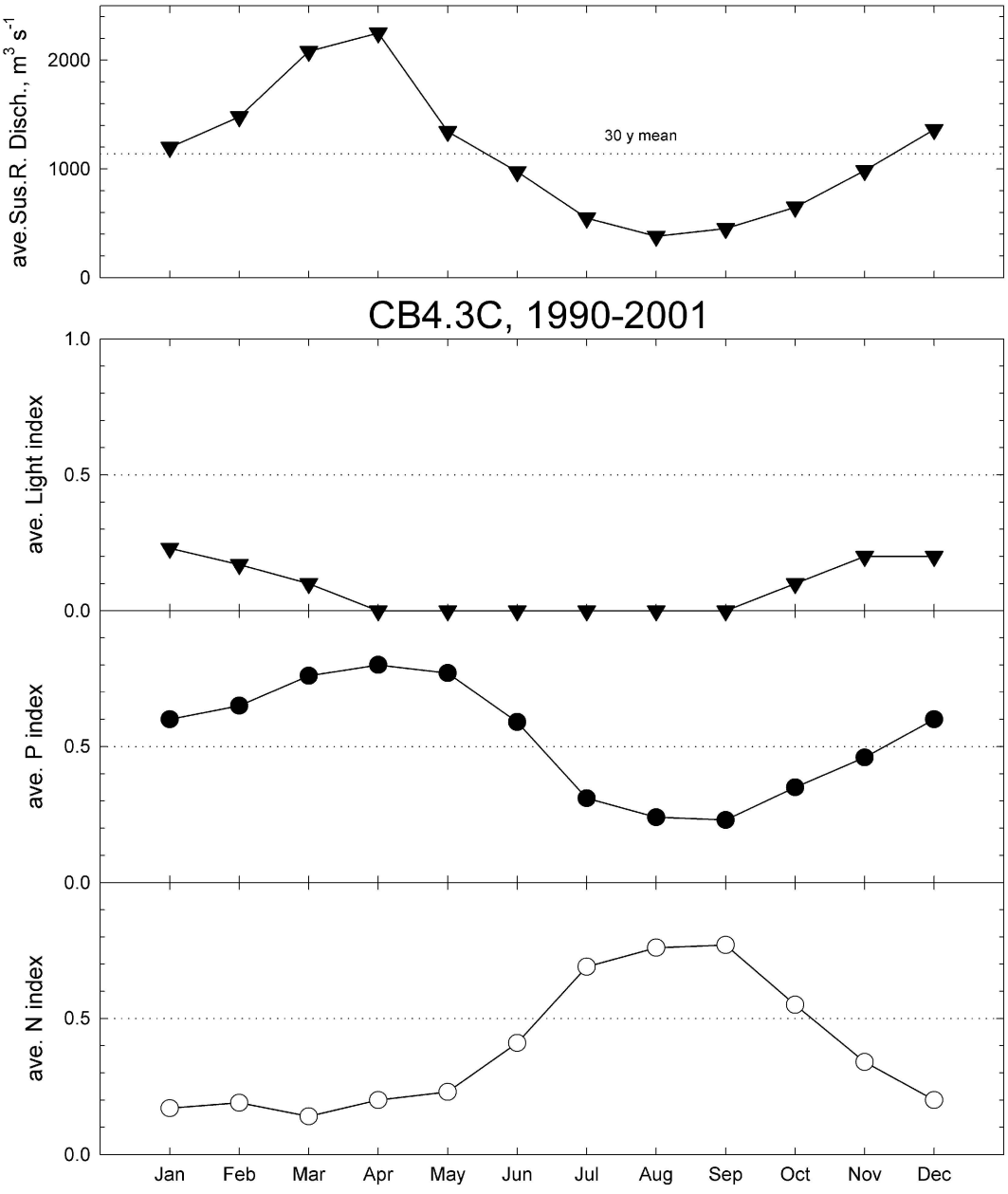

Fig. 6. Monthly summary of Light, P, and N indices for mesohaline station CB4.3C during 1990-2001. Values of the index > 0.5 indicate dominance by that resource. Upper panel is the average monthly Susquehanna River discharge during the same years, with the 30 year mean discharge shown as the horizontal line.

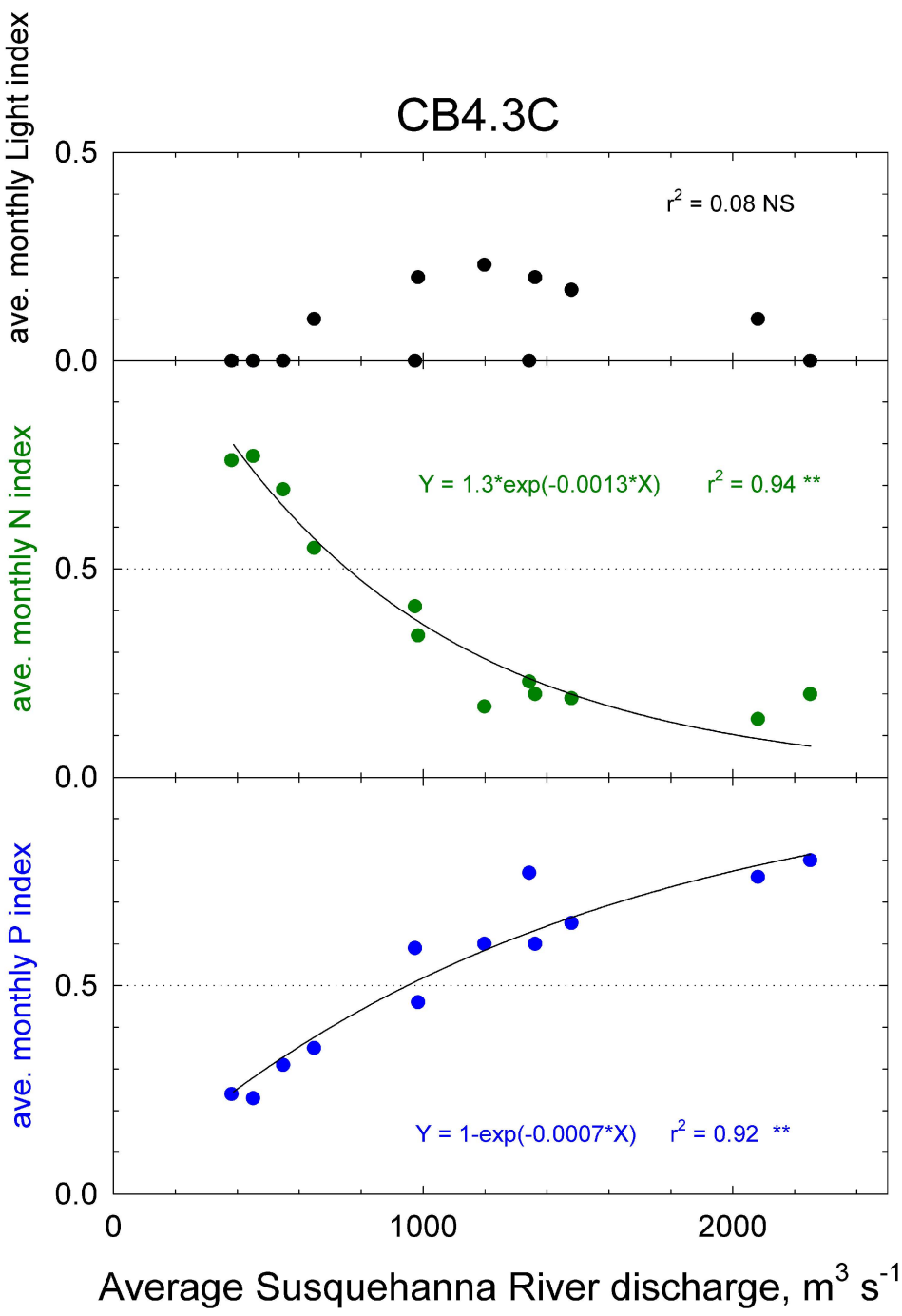

Fig. 7. Correlations between the average monthly indices and the average monthly river discharge for this station. The dotted line indicates an index of 0.5.

This is due to the high DIN/PO_4 in river discharge, which drives the plankton towards N sufficiency and P limitation. The seasonal changes shown in Fig. 6 therefore appear to be the result of low light and temperature in winter, large inputs of fresh water with high DIN/PO_4 in spring, large fluxes of PO_4 from sediments following the development of anoxia in bottom waters in late spring, and turnover (reaeration) of the water column in October (Fisher et al. 1992, Fisher et al. 1999).

The mesohaline Patuxent (LE1.1, Group B) was considerably different from the five mesohaline stations in Group A (compare Figs. 5 and 8). LE1.1 exhibited only a few significant responses to P in spring, and the N responses were larger, extended into the fall, and were nearly equivalent to the N+P additions. This station clearly is more N and less P limited throughout the year than the other mesohaline stations. Note the very large responses to N additions in Aug.-Sept. of 1998 (1000-1500 % responses relative to the control) and Sept. 2000 (800-900% of control). These large responses are indicative of extreme N limitation, which is probably the result of the (normal) seasonally low river inputs combined with decreasing wastewater inputs into the Patuxent and improving water quality. River inputs in August-September 1999 were considerably larger (the result of a wetter year plus a hurricane), which

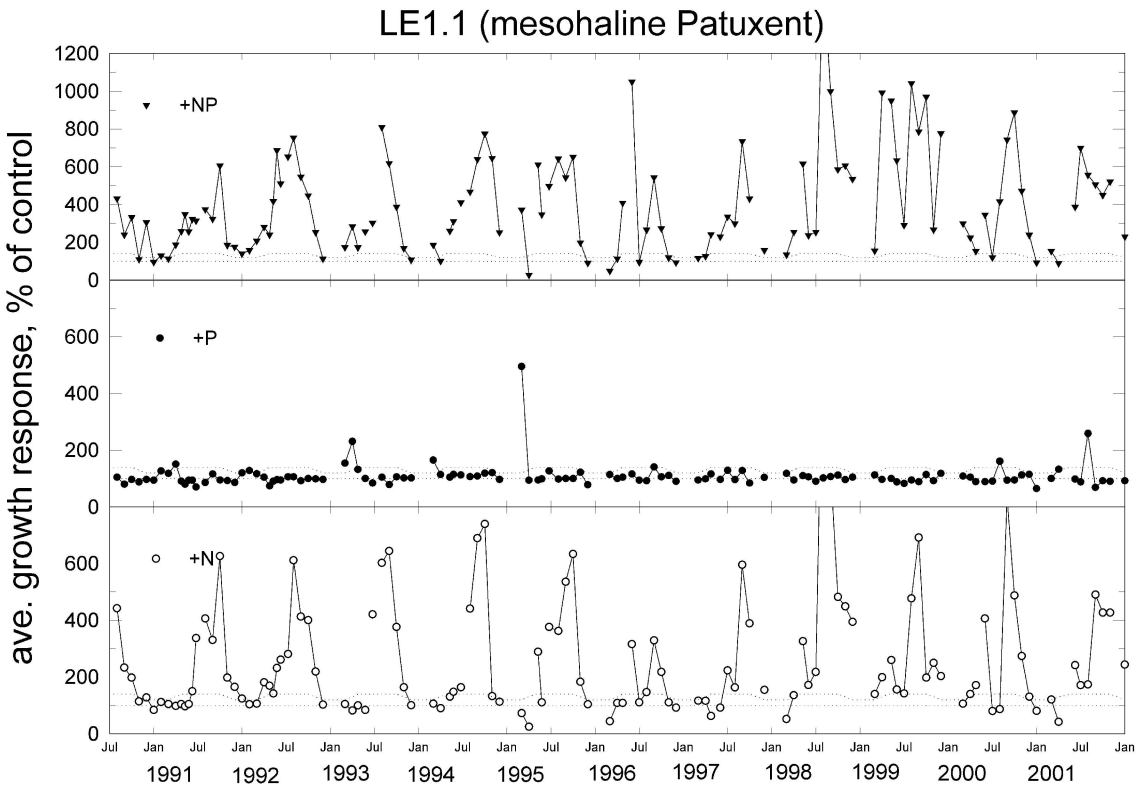

Fig. 8. Bioassay results at the mesohaline Patuxent station LE1.1. Straight dotted lines represent 100% of control (no response), and fluctuating dotted lines represent seasonally varying thresholds for significant responses. This station, the sole representative in Group B, has little response to P additions.

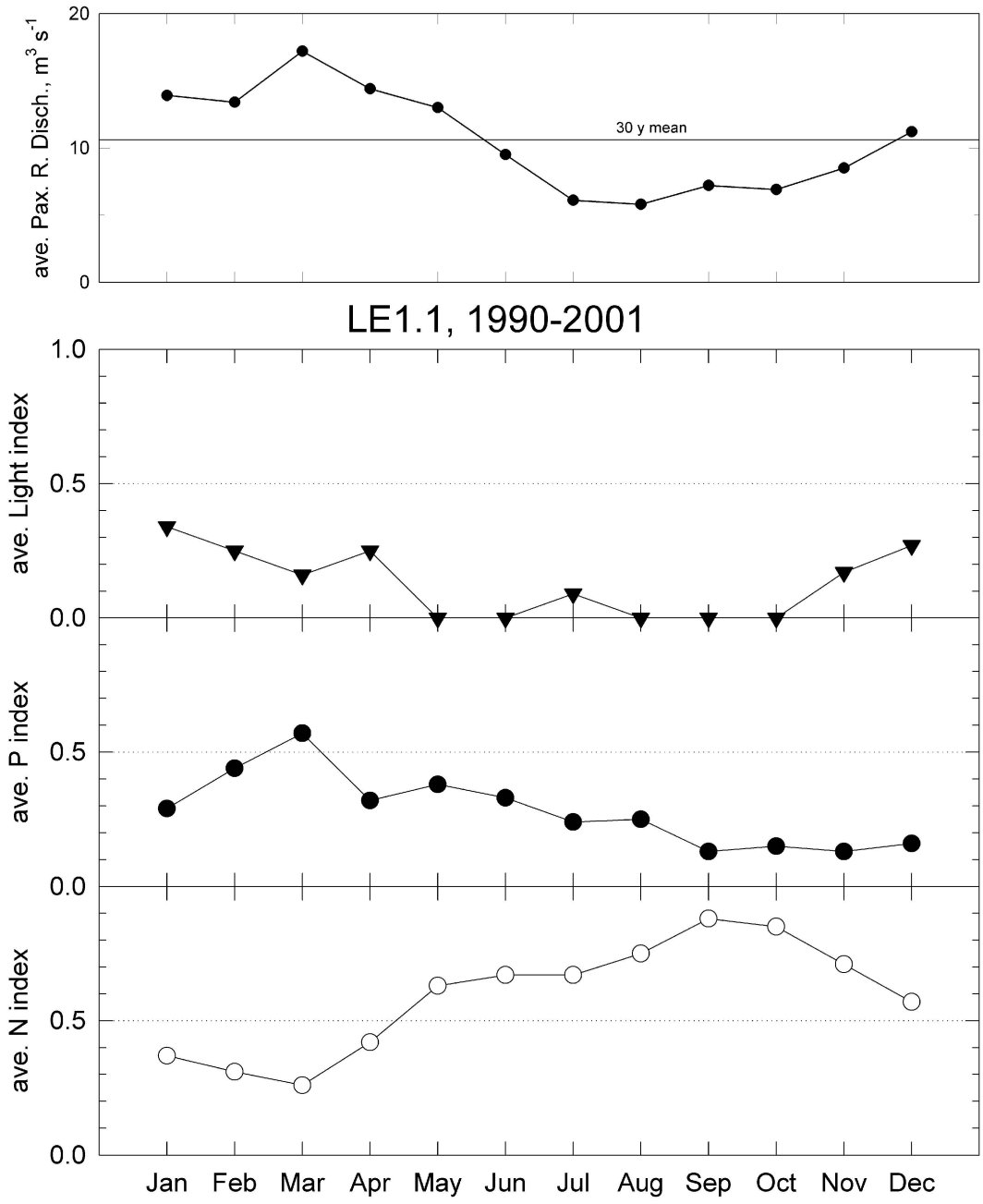

Fig. 9. Monthly summary of Light, P, and N indices for the Patuxent mesohaline station LE1.1 during 1990-2001. Values of the index > 0.5 indicate dominance by that resource. Upper panel is the average monthly Patuxent River discharge during the same years, with the 30 year mean discharge shown as the horizontal line.

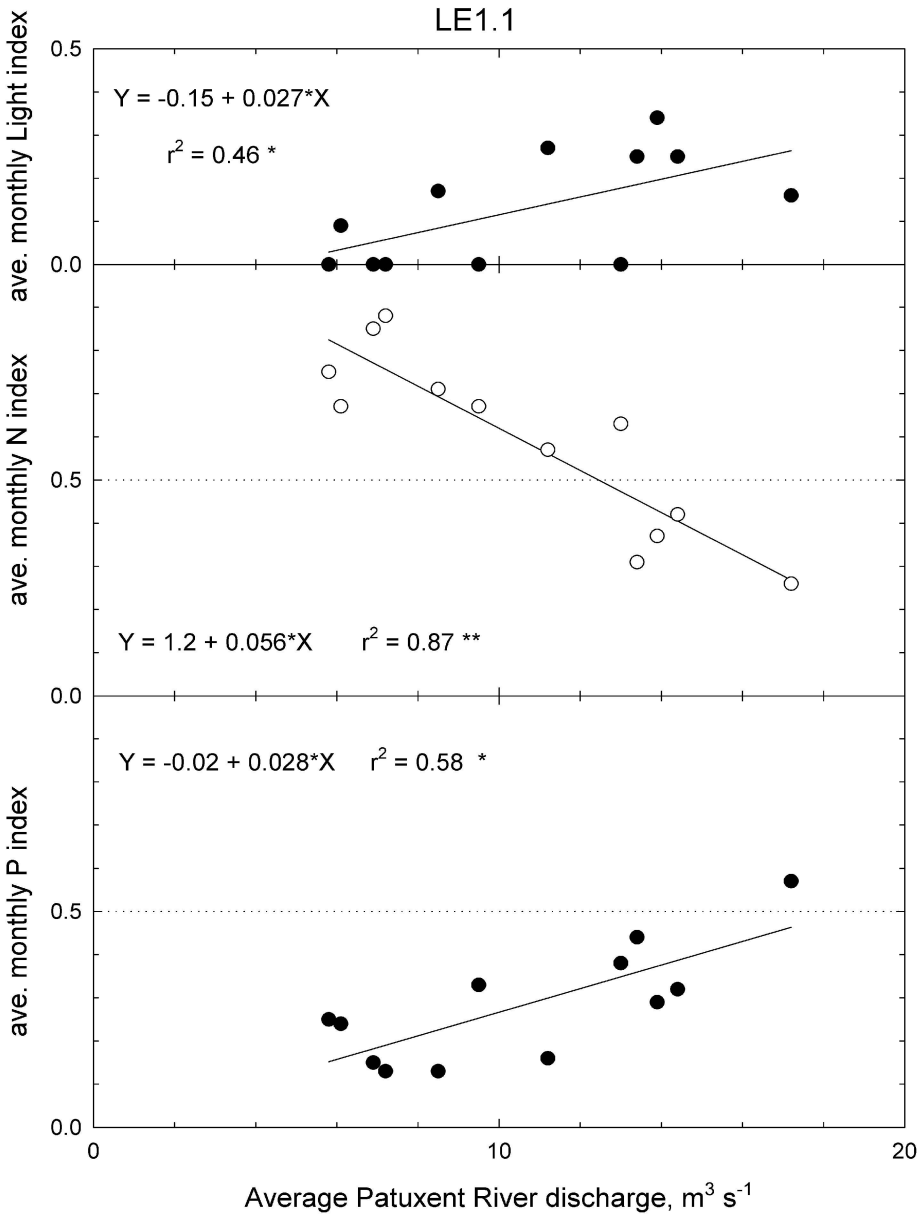

Fig. 10. Correlations between the average monthly indices and the average monthly river discharge for the Patuxent mesohaline station LE1.1. The dotted line indicates an index of 0.5.

probably reduced the severity of nutrient limitation in the summer of 1999.

The monthly summary of indices for the mesohaline Patuxent LE1.1 (Group B) is shown in Fig. 9. There is a much reduced role for P at this station (Group B) compared to the other mesohaline stations in Group A (Fig. 6). The increase in the P index in spring is due to the lack of N responses in spring, weak P responses, and large responses to N+P (see Fig. 8). The computation of the indices does not include the magnitude of the response, and the indices in Fig. 9 magnify the effect of the small responses to P in spring and dampen the large N responses in summer. However, the brief dominance of P in March at the highest flows and the longer dominance of N from May through December is clearly shown.

As at other mesohaline stations, there is a strong influence of river discharge on the monthly indices (Fig. 10). There are positive correlations between the P and light indices and a negative correlation between the N indices and discharge. The lesser importance of P at this station compared to the other mesohaline stations is probably due to sewage inflows with low N/P and lower seasonal variation of flow in the Patuxent (spring:summer = 3:1 compared to 6:1 in the Susquehanna; compare top panels of Fig. 6 and 9).

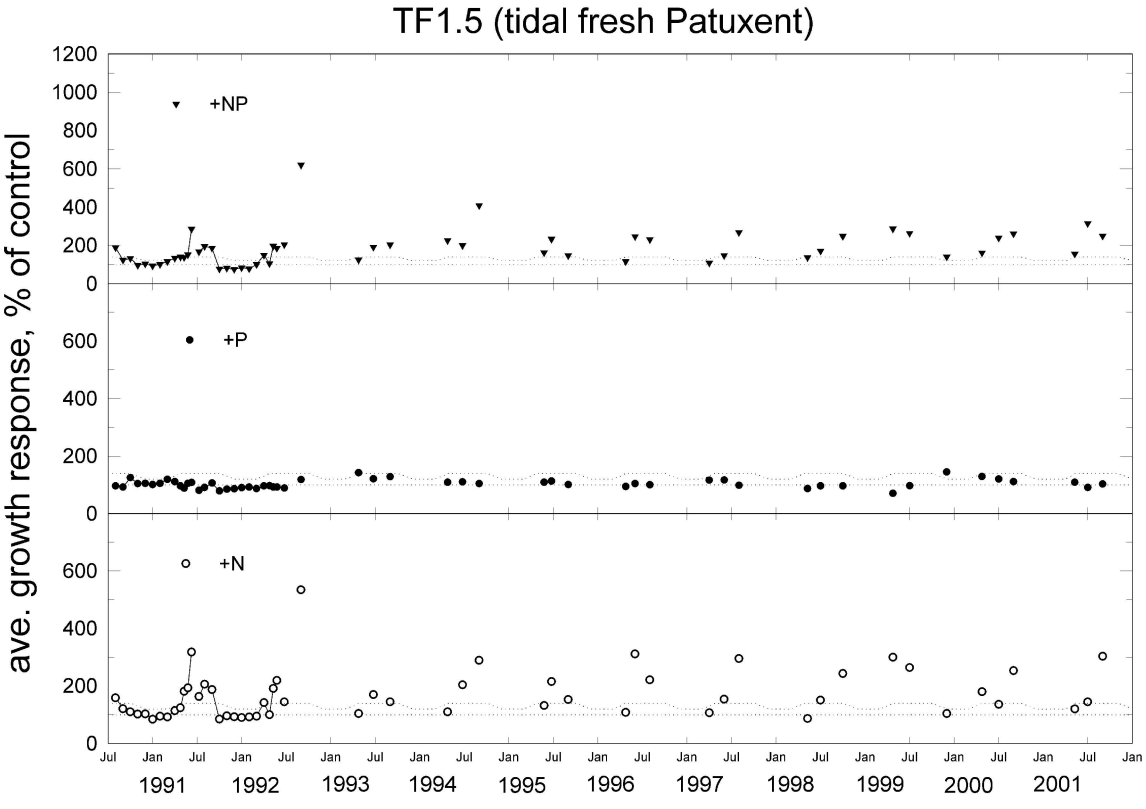

Fig. 11. Bioassay results at the tidal fresh Patuxent station TF1.5. Straight dotted lines represent 100% of control (no response), and fluctuating dotted lines represent seasonally varying thresholds for significant responses. This station, representing Group C responses, is more light limited than either Group A or B, exhibiting nutrient limitation primarily in summer.

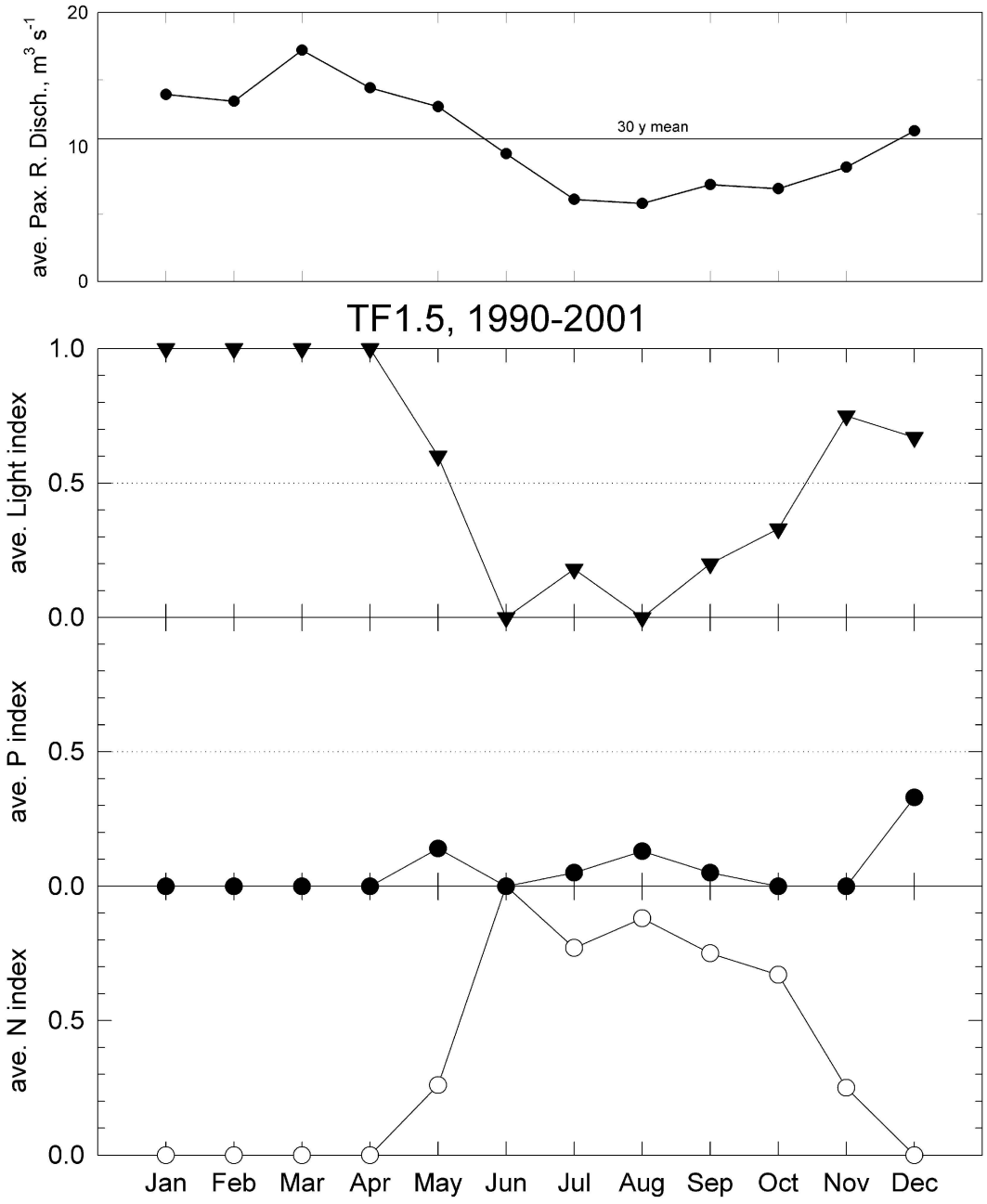

Fig. 12. Monthly summary of Light, P, and N indices for the Patuxent tidal fresh station TF1.5 during 1990-2001. Values of the index > 0.5 indicate dominance by that resource. Upper panel is the average monthly Patuxent River discharge during the same years, with the 30 year mean discharge shown as the horizontal line.

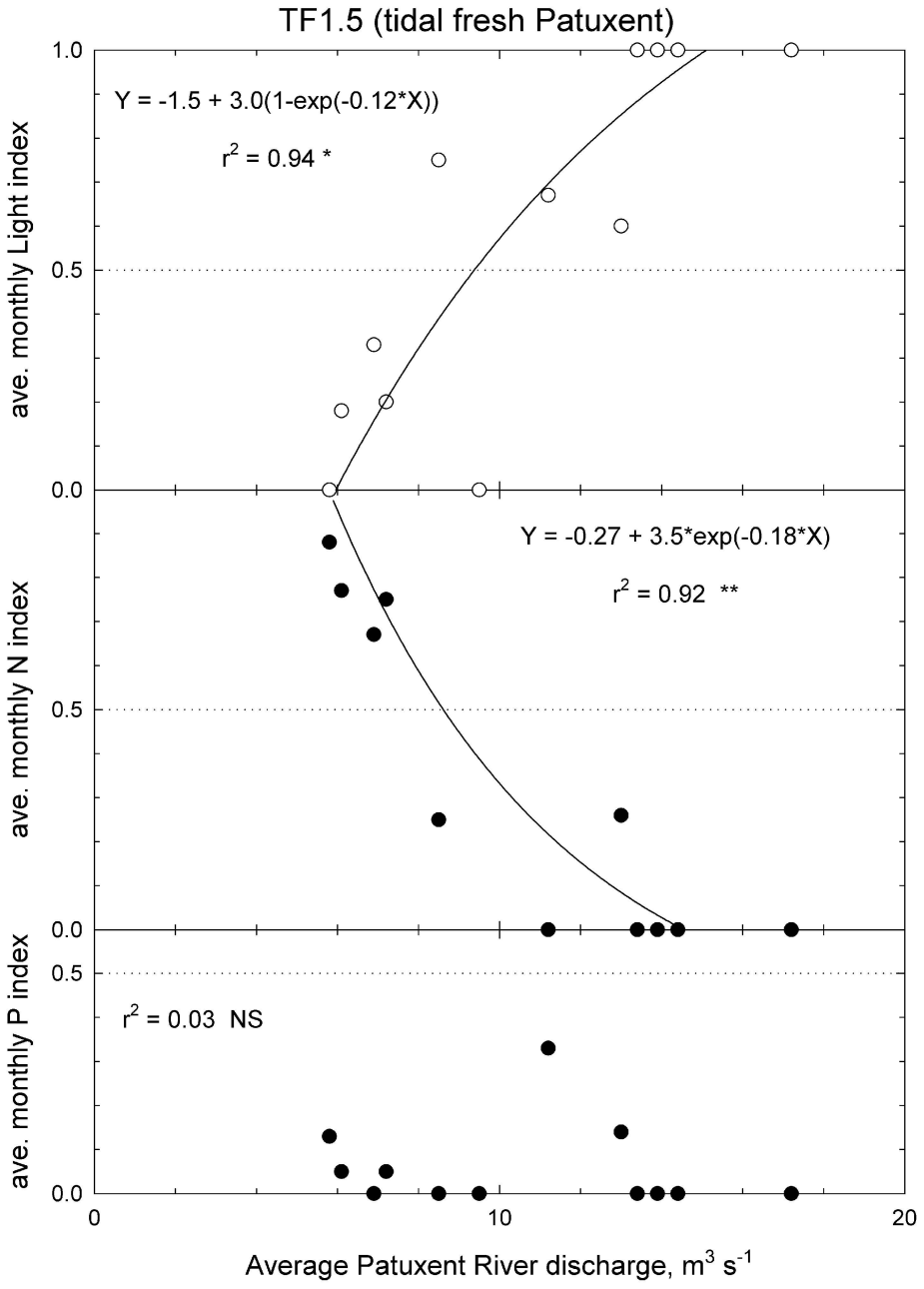

Fig. 13. Correlations between the average monthly indices and the average monthly river discharge for this station. The dotted line indicates an index of 0.5.

The three tidal fresh stations (Group C) were more light-limited and nutrient-saturated than the other two groups (see Fig. 11 for an example). These turbid, nutrient-rich stations showed few significant P or N responses, mainly in summer when DIN or PO_4 were depleted. These stations are sufficiently turbid and nutrient-rich that phytoplankton are primarily nutrient-saturated and light-limited, except during low flow, high temperature conditions.

The monthly summary of indices for the tidal fresh Patuxent station TF1.5 (an example of a Group C station) is shown in Fig. 12. This figure illustrates the dominant role of light limitation, except in the warmer months with low flow. Responses to added nutrients were observed only in summer (high temperatures and low flow) when N (Patuxent, Choptank) or P (Potomac) was depleted. The other nutrient played only a minor role during the summer months at these stations.

River discharge again appeared to be the primary control on the limiting resource (Fig. 13). At TF1.5 there was a strong inverse exponential relationship between the N index and a direct exponential relationship with the light index. High flows >12 $m^3 s^{-1}$ drive this station to light limitation, whereas lower flows allow the development of algal populations which reduce DIN concentrations until they become limiting. These stations represent small volumes and areas of Chesapeake Bay and are turbid, nutrient-rich transition zones with short water residence times. The dominance of light limitation for much of the year is expected in these regions.

The above figures describe the complex spatial and seasonal variation in resource limitation in Chesapeake Bay. Seasonally, high flows in spring with high DIN/PO_4 tend to create conditions for light (Group C) or P limitation (Group A, B). Lower flow conditions and high temperatures in summer usually result in N limitation at most stations. Spatially, most MD mesohaline stations exhibited similar responses (Group A), except for the mesohaline Patuxent (Group B), which showed greatly reduced responses to P additions. Tidal fresh stations (Group C) were generally turbid and nutrient-rich, responding only to nutrient additions in summer under low flow conditions.

Interannual Trends of nutrient-addition bioassays

There are some interannual trends in the bioassay results. Trends at the three stations described above as examples of each station group are shown in Figs. 14-16. We tested for trends by using annual averages of monthly indices in order to integrate over the strong seasonal variations in resource limitation. Double sampling in some months (e.g., May) were averaged for the month, and occasional missing values were interpolated between adjacent months. At tidal fresh stations, which are only sampled three times per year (May, July, and September), the annual average was the average of the three observations. At main Bay station CB4.3C, representative of the five similar mesohaline stations in Group A, there was a clear reduction in light limitation during the early 1990's, although a linear trend was not significant. P indices were stable, except for lower values in 1990 (a partial data year), and there have been slow but steady increases in N limitation over the entire period. P was the dominant limiting resource during 1993-1996, perhaps due to the predominance of wet years during this period

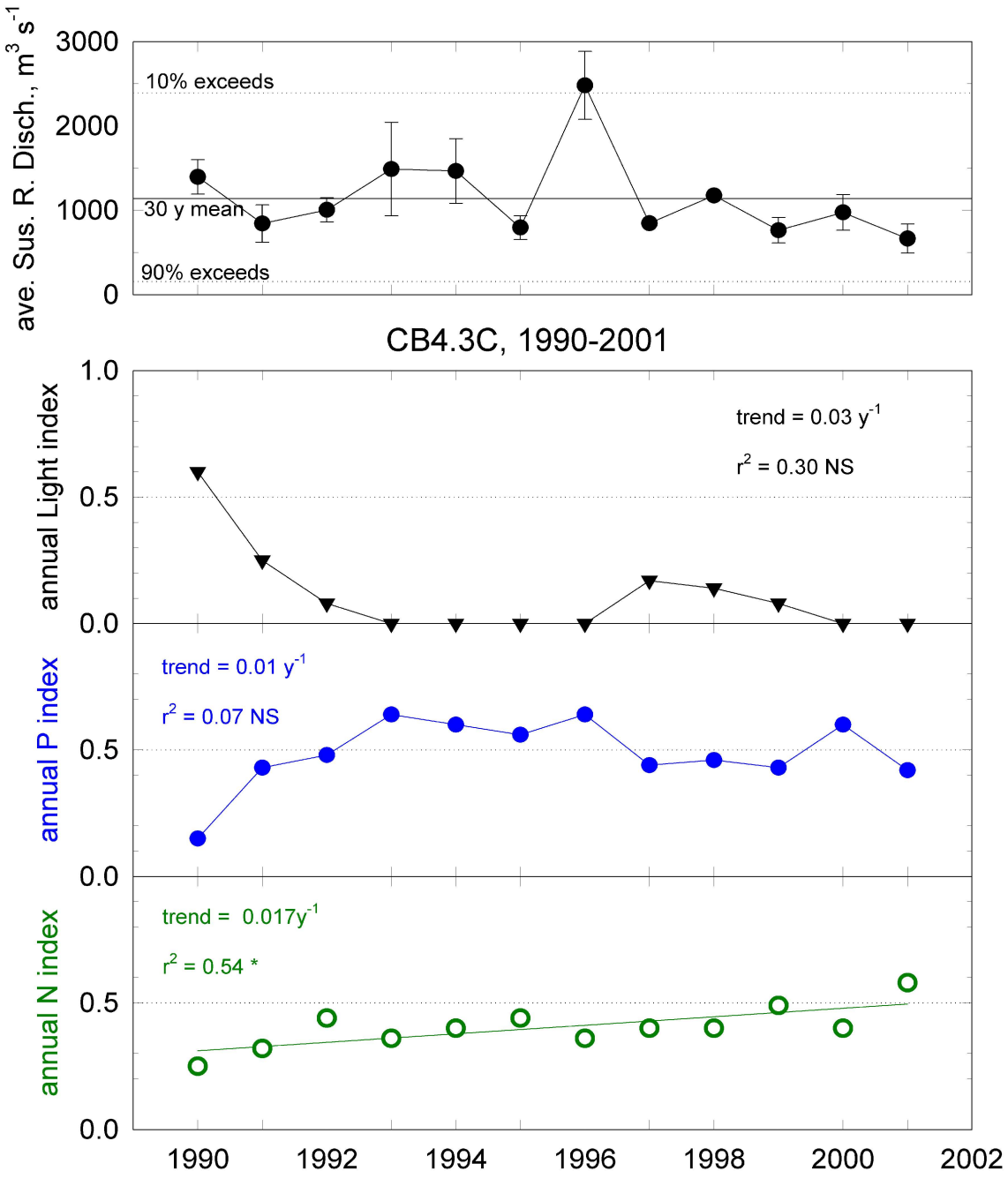

Fig. 14. N, P, and Light indices at mesohaline station CB4.3C during calendar years 1990-2001. Indices > 0.5 indicate the dominant resource limiting phytoplankton growth in each year. Discharge data in top panel from USGS.

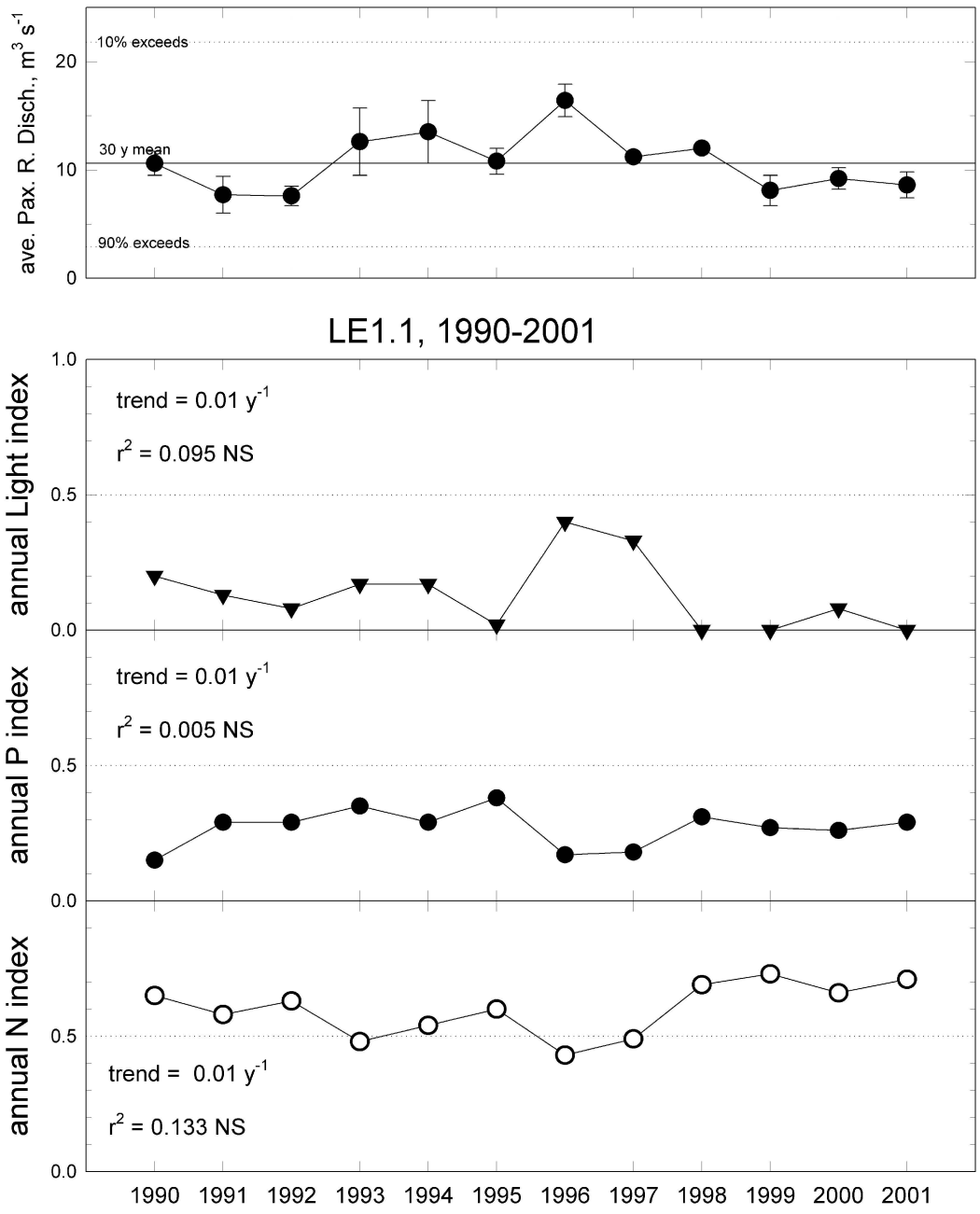

Fig. 15. N, P, and Light indices at the Patuxent mesohaline station LE1.1 during calendar years 1990-2001. Indices > 0.5 indicate the dominant resource limiting phytoplankton growth in each year. Discharge data in top panel are from USGS.

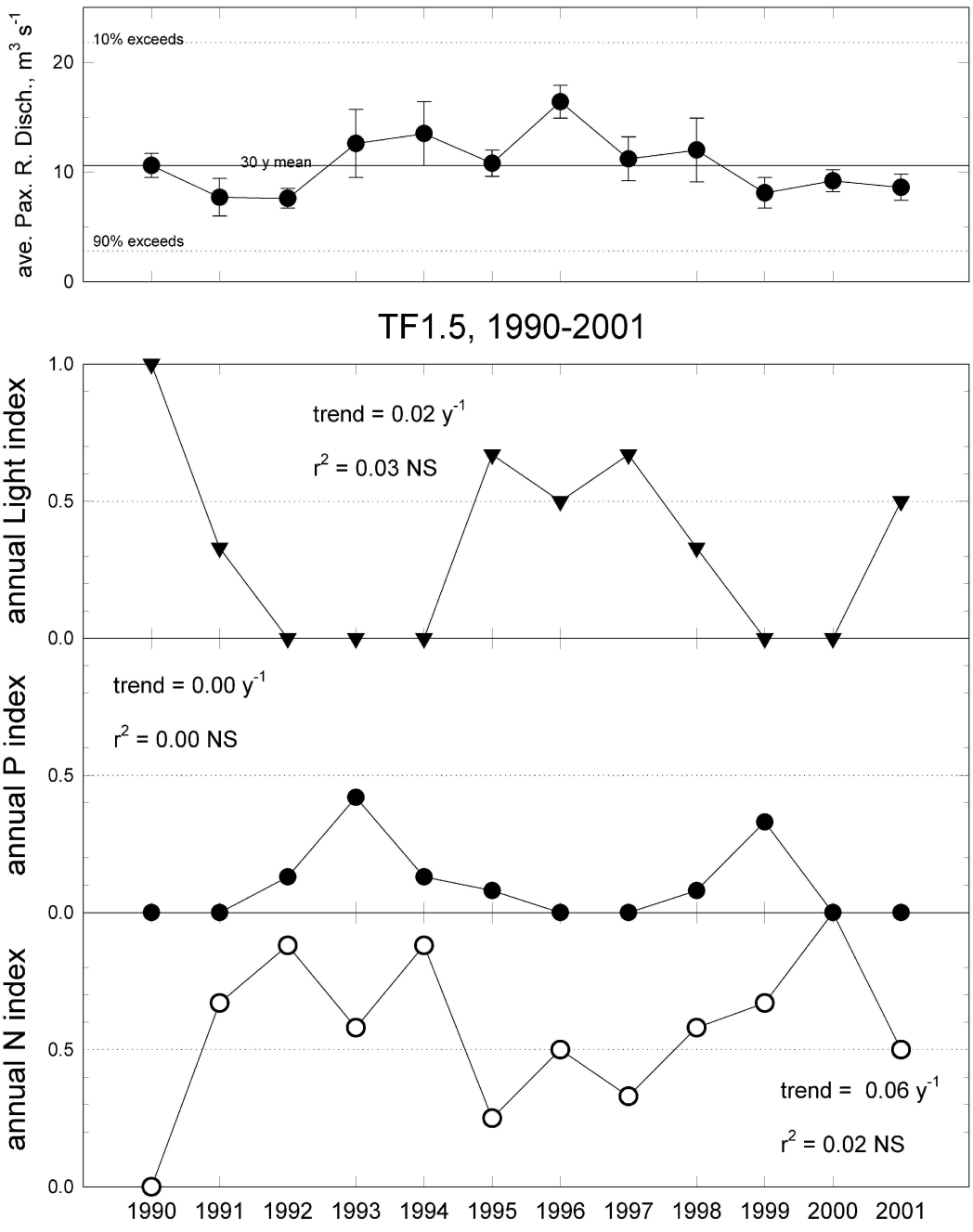

Fig. 16. N, P, and Light indices at the Patuxent tidal fresh station TF1.5 during calendar years 1990-2001. Indices > 0.5 indicate the dominant resource limiting phytoplankton growth in each year. Discharge data in the top panel are from USGS.

(see top panel of Fig. 14). In contrast, at the Patuxent mesohaline station (LE1.1, Fig. 15), there were no significant trends in the annual N, P, or light indices over the period, although after 1998 we have obtained the highest annual N indices (~0.7) and lowest light indices (0.0-0.1) that we have recorded at this station. Some of the interannual variations at the mesohaline stations were correlated with river flow (e.g., CB4.3 but not LE1.1), and the relationships were similar to those observed at the monthly time scale, except that they were statistically weaker (see Table 6).

Tidal fresh stations showed considerably more interannual variability than the mesohaline stations. At the Patuxent and Potomac tidal fresh stations, there were large variations but no significant interannual trends in indices (see Fig. 16 for an example from Patuxent tidal fresh station TF1.5); however, at the Choptank tidal fresh station, the annual N index significantly decreased and the annual P index significantly increased over the time period. Interannual variations in the indices at the tidal fresh stations were not correlated with interannual variations in river flow (Table 6).

The relationships between the indices and river discharge are summarized in Table 6. All significant relationships between N indices and flow at the monthly and interannual time scale were negative, whereas all significant relationships between P indices and discharge were positive (see Figs. 7, 10, 13 for examples). There were stronger statistical connections at the monthly time scale than at the annual scale, probably due to larger seasonal changes in flow compared to changes at the interannual time scale. These relationships indicate that the high inflows of turbid, N-rich river water in winter and spring promote either light-limited or P-limited algal growth and that the lower flows of summer promote N limitation.

Diagnostic Indicators of Resource Limitation

We have identified several diagnostic indicators which are correlated with resource limitation. In general, the mean values of the indicators significantly differ between classes of bioassay results. We have examined extracellular dissolved inorganic N (DIN), extracellular phosphate (PO_4), the ratio of DIN/PO_4, molar particulate ratios (POC/PP, PN/PP), intracellular amino acid ratios (glutamine/glutamate, gln/glu), and alkaline phosphatase activity (Alk. Pase). Summary statistics are shown in Table 7. In separate reports sent to DNR in previous years, we summarized the statistical relationships between these and other indicators and the bioassay classes, including fitted frequency distributions for each indicator which permit calculations of the probability of N, P, and light limitation (Fisher et al. 1999a, b). Furthermore, in collaboration with Dr. Elgin Perry, we have developed an improved statistical model which predicts the probability of N, P, and Light limitation.

Two indicators of N limitation are shown in Fig. 17. Dissolved inorganic N (DIN = $NH_4 + NO_2 + NO_3$) is an excellent predictor of responses in the bioassays. EXN and PRN bioassay responses occurred at low DIN, and concentrations of DIN <5 µM (0.07 mg DIN ℓ^{-1}) are a good predictor of N responses. At higher DIN, P and light limitation (nutrient saturation) were observed. Because of the utility of DIN to predict both N and P limitation, DIN was an

Table 6. Summary of relationships between indices of resource limitation and local river flows and interannual trends (last two columns). Stations are grouped by region, from fresh to mesohaline. Statistical parameters are given for the relationships between monthly average indices and monthly average flows as well as annual average indices and annual average flow and year over calendar years 1991-2001. Calendar year 1990 was excluded because it is a partial year beginning in August. All significant relationships between N indices and flow are negative, and all significant relationships between P and light indices and river flow are positive, indicating suppression of N limitation and creation of the potential for P or light limitation by the high N content and turbidity of inflowing river waters. Relationships between indices and flow are stronger at the monthly time scale than at the annual time scale. Most interannual trends of indices were not significant, except at upper Bay station CB3.3C and tidal fresh Choptank ET5.1, which exhibited significantly more P limitation and less light or N limitation (CB3.3C data only through 1999). CB4.3 showed significantly more N limitation over time.

			Monthly			Interannual				
region	station	index	relationship to flow	best model	r^2	relationship to flow	best model	r^2	interannual trends	r^2
main bay	CB3.3C	N	negative	exponential	0.927 **	none	none	0.009 NS	none	0.09 NS
		P	none	none	0.232 NS	none	none	0.244 NS	pos.	0.47 *
		Light	positive	linear	0.358 *	none	none	0.149 NS	neg.	0.40 *
	CB4.3C	N	negative	exponential	0.942 **	none	none	0.152 NS	pos.	0.54 **
		P	positive	exponential	0.920 **	positive	linear	0.465 *	none	0.07 NS
		Light	none	none	0.078 NS	none	none	0.205 NS	none	0.30 NS
	CB5.2	N	negative	exponential	0.929 **	negative	linear	0.727 **	none	0.29 NS
		P	positive	linear	0.430 *	positive	linear	0.382 *	none	0.14 NS
		Light	none	none	0.141 NS	none	none	0.058 NS	neg.	0.72 **
Choptank	ET5.1	N	negative	exponential	0.706 **	none	none	0.019 NS	neg.	0.49 *
		P	none	none	0.260 NS	none	none	0.092 NS	pos.	0.46 *
		Light	positive	exponential	0.733 **	none	none	0.136 NS	none	0.07 NS
	ET5.2	N	negative	exponential	0.827 **	none	none	0.128 NS	none	0.10 NS
		P	none	none	0.328 NS	none	none	0.238 NS	none	0.05 NS
		Light	positive	linear	0.707 *	none	none	0.193 NS	none	0.00 NS
Patuxent	TF1.5	N	negative	exponential	0.916 **	none	none	0.010 NS	none	0.06 NS
		P	none	none	0.030 NS	none	none	0.006 NS	none	0.00 NS
		Light	positive	exponential	0.943 **	none	none	0.087 NS	none	0.06 NS
	LE1.1	N	negative	linear	0.816 **	none	none	0.372 *	none	0.13 NS
		P	positive	linear	0.598 *	none	none	0.072 NS	none	0.00 NS
		Light	positive	linear	0.397 *	none	none	0.221 NS	none	0.09 NS
Potomac	TF2.3	N	none	none	0.102 NS	none	none	0.076 NS	none	0.20 NS
		P	none	none	0.163 NS	none	none	0.049 NS	none	0.00 NS
		Light	none	none	0.174 NS	none	none	0.059 NS	none	0.02 NS
	LE2.2	N	negative	exponential	0.941 **	none	none	0.167 NS	none	0.03 NS
		P	positive	exponential	0.562 *	none	none	0.123 NS	none	0.04 NS
		Light	none	none	0.253 NS	none	none	0.001 NS	none	0.06 NS

Table 7. Statistics on indicators of resource limitation in Chesapeake Bay for the seven bioassay classifications and for all observations for the period Jul.1992 - Dec. 2001. The mean and standard errors were computed for untransformed data. (* = gln/glu was not analyzed after June 1998).

indicator	statistic	EXN	PRN	BNP	PRP	EXP	INC	NOR	ALL
DIN	n	60	202	84	134	33	37	70	665
	minimum	0.0	0.0	0.0	0.5	0.9	0.0	0.5	0.0
	maximum	7.1	9.8	16.5	171.5	143.7	99.5	283.3	283.3
	mean	1.1	1.3	4.7	33.7	45.2	20.3	43.5	18.3
	std. error	0.2	0.1	0.4	2.3	6.0	4.3	5.2	1.1
gln/glu*	n	13	29	20	23	13	1	21	127
	minimum	0.05	0.06	0.07	0.04	0.06	0.13	0.00	0.00
	maximum	0.69	0.61	1.44	1.25	10.58	0.13	2.97	10.58
	mean	0.19	0.20	0.35	0.48	1.82	0.13	0.49	0.50
	std. error	0.05	0.02	0.08	0.07	0.79	-	0.14	0.09
POC/PP	n	37	86	43	70	24	27	44	349
	minimum	40.2	42.9	45.7	67.9	48.4	34.8	43.6	34.8
	maximum	133.1	210.1	391.5	332.2	246.5	487.1	212.0	487.1
	mean	82.4	98.0	116.9	134.7	133.1	131.0	104.7	111.9
	std. error	3.4	3.3	8.8	5.8	11.0	18.8	6.1	2.8
PN/PP	n	37	86	43	70	24	27	44	349
	minimum	5.7	5.7	8.1	8.9	7.2	5.5	5.2	5.2
	maximum	21.3	27.2	59.8	43.6	29.4	53.3	30.5	59.8
	mean	11.3	13.7	17.4	17.8	17.7	16.8	14.6	15.4
	std. error	0.5	0.4	1.2	0.7	1.1	1.8	0.9	0.3
DIN/PO_4	n	60	202	84	134	33	37	70	665
	minimum	0.0	0.0	0.4	1.4	9.8	0.0	1.1	0.0
	maximum	65.0	508.7	1648.	9999	4073.	2378	4268	9999
	mean	8.8	30.6	150.9	1417.9	890.1	384.6	484.7	479.2
	std. error	1.8	4.9	27.5	208.4	173.3	95.5	94.4	44.8
AP/chla	n	58	196	84	134	32	36	70	655
	minimum	0.3	0.4	0.8	0.7	0.8	1.2	0.1	0.1
	maximum	41.9	90.9	173.4	170.2	244.1	237.9	75.0	244.1
	mean	4.7	11.1	21.6	26.0	22.9	22.1	10.7	16.0
	std. error	0.9	1.0	3.0	2.6	7.9	7.1	1.9	1.0
PO_4	n	60	202	84	134	33	37	70	665
	minimum	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	maximum	1.41	1.80	0.30	1.04	1.23	1.12	1.50	1.80
	mean	0.32	0.16	0.07	0.08	0.19	0.12	0.28	0.16
	std. error	0.04	0.02	0.01	0.01	0.05	0.03	0.04	0.01

important parameter in the statistical model developed with Elgin Perry. The intracellular amino acid ratio is also a good predictor of N limitation (top panel, Fig. 17). Molar values of intracellular glutamine/glutamate increase approximately exponentially from EXN to EXP, and values <0.5 (the Flynn index, Flynn et al. 1989, 1993, 1994) can be used to predict N stress (including PRP responses, where N is almost in short supply); for Chesapeake Bay, values <0.3 are better predictors of N-responding bioassays (EXN, PRN). Due to the expense, we no longer obtain data on this measurement.

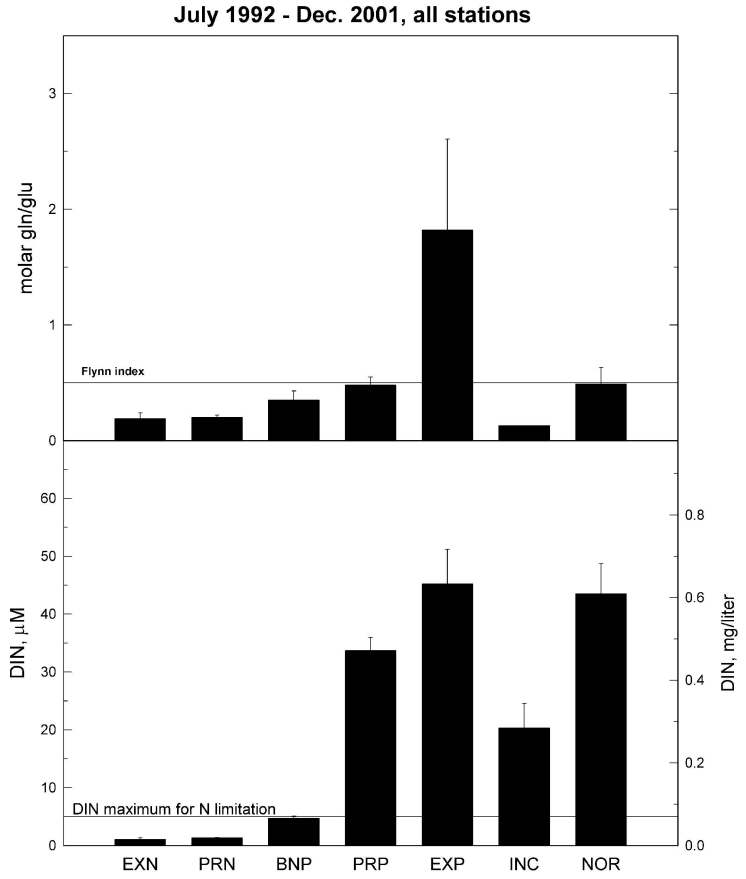

Fig. 17. Indicators of N limitation. Mean and standard error of dissolved inorganic N (DIN) and intracellular amino acid ratios (glutamine/glutamate) in Chesapeake Bay as a function of bioassay response type.

Particulate and nutrient ratios can be used to identify both N and P limitation (Fig. 18). Low values of all three ratios were associated with N responses in bioassays, and high ratios occurred under P limitation. Light limitation often occurred when particulate ratios wereclose to Redfield stoichiometry. Note that the two particulate ratios corresponded closely to Redfield elemental proportions, and the mean values within each bioassay classification deviated by less than a factor of 2 from 106:1 and 16:1. However, DIN/PO_4 associated with P limitation greatly exceeded the Redfield stoichiometry of 16:1. EXP and PRP responses in the bioassays occurred at very high molar ratios of DIN/PO_4, generally >500:1 (excess DIN and depleted PO_4 - see Table 7), probably because of the use of intracellular poly-PO_4 reserves as a source of P. In fact, the general pattern of response in Fig. 18 is the result of inflexible C and N content and varying P storage (Cembella et al. 1984). There is normally intracellular storage of poly-phosphates in P-sufficient plankton, and depletion of poly-phosphate stores in P-deficient plankton. Intracellular poly-PO_4, perhaps normalized to PP, is another potential indicator of P limitation, but we have not investigated this parameter.

P limitation was readily identified by high DIN/PO_4 and CNP ratios (Fig. 18), as well

as high alkaline phosphatase activity (Fig. 19). Rates of alkaline phosphatase activity in excess of 10 nmol PO_4 µg $chla^{-1}$ h^{-1} were associated with P responses; a similar level of activity (5 nmol PO_4 µg $chla^{-1}$ h^{-1}) was first suggested in culture studies of freshwater algae by Healey and Hendzel (1979), but our data suggest somewhat higher values for Chesapeake Bay. Our light-limited bioassays (NOR) and N bioassays (EXN, PRN) exhibited mean activity levels of ~5-10 nmol PO_4 µg $chla^{-1}$ h^{-1}, while bioassays showing P responses had means >20 nmol PO_4 µg $chla^{-1}$ h^{-1}. Furthermore, the inconclusive bioassays (INC) also had high activities equivalent to BNP, PRP, and EXP responses, suggesting severe P deficiency and possible collapse of the control as a reason for the INC classification. Note that INC bioassays also had high particulate and nutrient ratios as well (Fig. 18).

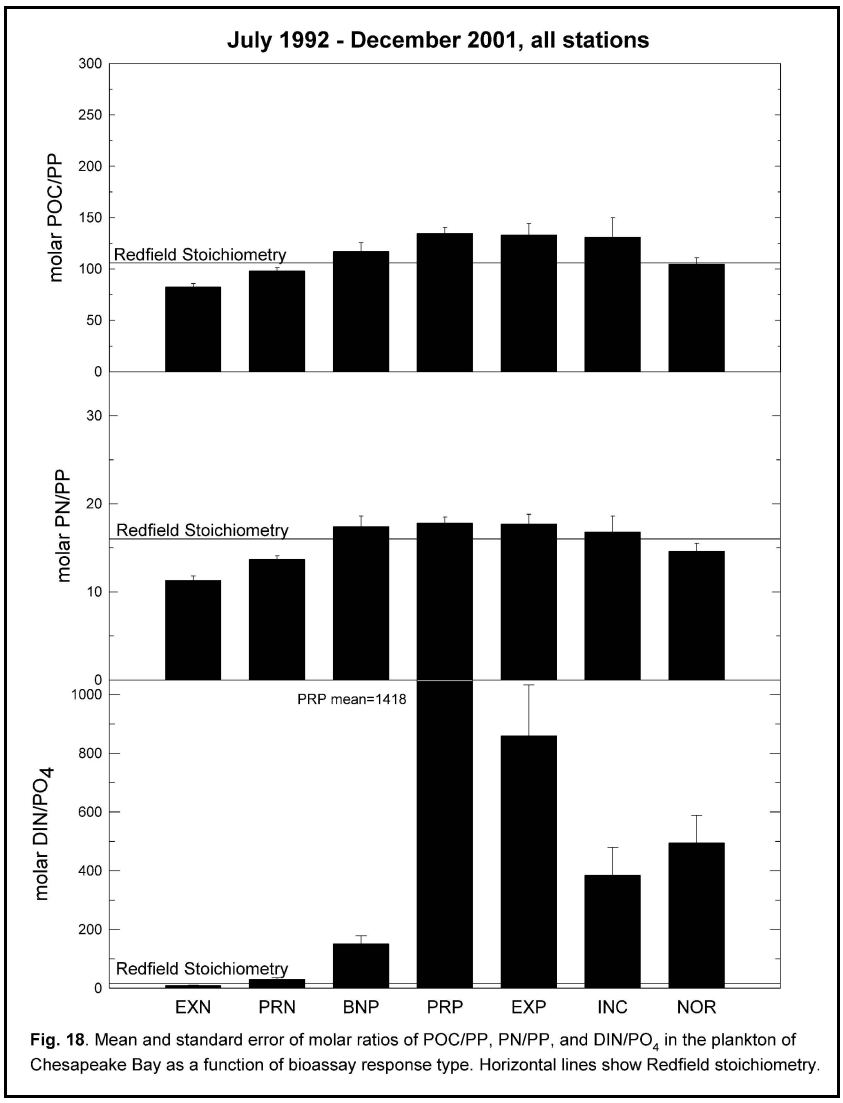

Fig. 18. Mean and standard error of molar ratios of POC/PP, PN/PP, and DIN/PO_4 in the plankton of Chesapeake Bay as a function of bioassay response type. Horizontal lines show Redfield stoichiometry.

Extracellular phosphate concentration was not a useful indicator of P limitation (Fig. 19). The highest average concentrations of PO_4 were associated with EXN, EXP, and NOR bioassays, indicating little predictability from PO_4 concentration alone. Note that the averages of all concentrations were quite low (0.1-0.3 µM or 0.002-0.009 mg PO_4-P ℓ^{-1}, see Table 7) and close to the colorimetric detection limit of ~0.02 µM (0.001 mg PO_4-P ℓ^{-1}), regardless of bioassay response. This suggests little excess P in Chesapeake Bay, even under N or light limitation, but also little predictive power for PO_4 concentration as an indicator of P limitation.

Resource-addition bioassays

We introduced the use of resource-addition bioassays in order to refine the border between light and nutrient limitation in Chesapeake Bay. Light limitation is common in the Bay

in winter and in most tidal fresh areas throughout the year, except in summer (Fisher et al. 1999). In the past we have inferred light limitation from equal growth responses in the control and nutrient addition treatments at 58 % ambient light (e.g., Fig. 3); however, not all bioassays classified as "NOR" are as clear as the example in Fig. 3, and some, particularly at low temperatures, may be misclassified, primarily because responses in winter are smaller than in summer and sometimes do not exceed experimental errors (Table 3). Therefore, we designed the resource-addition bioassay described in Table 2 to obtain more detailed information on light limitation, and the first resource addition bioassays were done in July 2000. In the coming year, we will continue this new approach, with some modifications based on the results from July 2000 - December 2001, which we describe below.

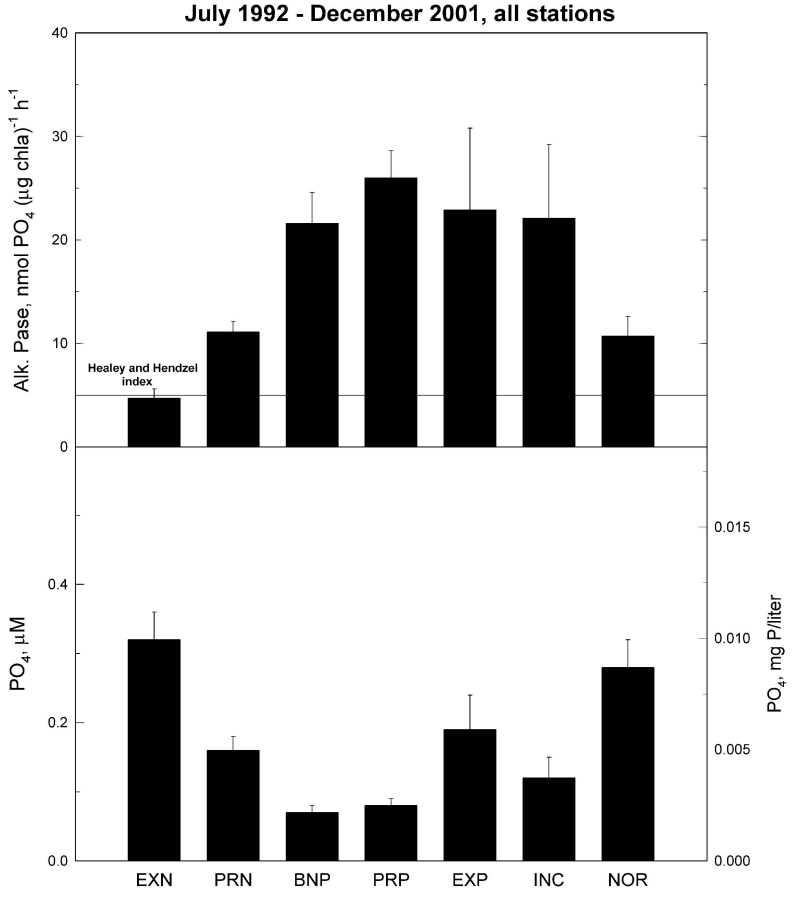

Fig. 19. Mean and standard error of dissolved reactive P (PO_4) and alkaline phosphatase activity per unit chlorophyll in Chesapeake Bay as a function of bioassay response type.

The resource-addition bioassays are expanded versions of the nutrient-addition bioassays. The treatments of the nutrient addition bioassay (duplicate control, +N, +P, +NP; all at 58 % light) are included, but there is also a time series at the 58 % light level (day 1, 2, 3, 5). We have added this to show the time course of the responses to the added nutrients. In addition, we also included a light series (6%, 11%, 20%, 34%, 58%, 100%) for each nutrient addition, sampled only on the last day, to show the effects of light on algal responses. The time series uses chlorophyll a and nutrient concentrations to illustrate nutrient depletion and algal accumulation during the bioassays (except at the end points when C fixation is also measured), and the light series shows algal growth responses (chlorophyll a and C fixation) as a function of available light during the incubation and nutrient additions. Although we did not "add" light, as we did for N and P, we manipulated light as a resource available for algal growth by screening incubations

down to 6 % of surface irradiance. Examples of the resource-addition bioassays are reported here as a time series at the 58 % light level and as a light series after 5 days.

We have performed ten resource-addition bioassays between July 2000 and May 2002 (Table 8). The initial resource-addition bioassays were done during several seasons and at a variety of stations, but we are now concentrating on winter months and tidal fresh stations. The four bioassays done in summer (July - Sept, >20°C, low N conditions) responded consistently to N additions, and a main bay sample (CB4.3) for May 2002 is expected to respond primarily to P additions. These were primarily done to establish the resource-addition bioassay method and to compare the results with our nutrient-addition bioassays described above. However, the main application of the resource-addition bioassays is in winter and at tidal fresh and oligohaline stations where we have primarily observed light limitation (NOR) in nutrient-addition bioassays. Below we describe the Dec. 2001 resource-addition bioassay at Choptank tidal fresh station ET5.1 and the Jan. 2001 resource-limitation bioassay at LE1.1 as examples of this new approach.

Table 8. Listing of resource-addition bioassays used to assess resource (light, N, and P) limitation in Chesapeake Bay. Resource-addition bioassays are done at varying light levels and varying nutrient additions, along with a time series in the 58 % and 11% light incubations. In each bioassay, the original water sample is processed for initial conditions and subdivided into treatments (see Table 2).

date	station	type	temp	µM DIN	µM PO_4	Response
July 2000	TF1.5	PaxTF	26.5	15.1	0.93	rapid DIN uptake, PRN
Aug.2000	LE1.1	PaxMeso	26.0	0.5	0.37	depleted DIN, EXN
Sep.2000	TF1.5	PaxTF	21.0	0.4	0.38	depleted DIN, EXN
Jan.2001	LE1.1	PaxMeso	1.8	1.3	0.01	depleted DIN, no uptake, grazing?
May2001	TF1.5	PaxTF	21.3	41.2	0.33	excess DIN, NOR, high chla
Sep.2001	ET5.1	ChopTF	25.9	20.6	0.16	excess DIN, NOR, high chla
Dec.2001	ET5.1	ChopTF	11.2	101.7	0.41	excess DIN, NOR
Jan.2002	CB2.1	BayTF	1.8	58.9	0.01	not yet available
Apr.2002	ET5.2	ChopMes	14.8	3.7	0.01	not yet available
May2002	CB4.3	BayMeso	17.0	13.3	0.02	not yet available

The Dec. 2001 resource-addition bioassay done with water from the Choptank tidal fresh station ET5.1 is an example of a light-limited response. At this time of year with low levels of light, incubating a sample from this station at the 11% light level is essentially equivalent to keeping the samples in the dark (Fig. 20). There was essentially no assimilation of the added NH_4 and PO_4, and chlorophyll a did not increase, despite the high nutrient levels. In contrast, when we incubated at the 60% light level, we induced a bloom (Fig. 21). Nutrients declined, and chlorophyll a increased in all treatments, including the control. Because of the high ambient concentrations (DIN > 100 µM or 1.4 mg N L^{-1}, PO_4 > 0.4 µM or 0.012 mg PO_4-P L^{-1}), there were no differential responses to any treatment. Everything grew, including the control. This example clearly illustrates a time series of our concept of a nutrient-saturated, light-limited response developed from earlier nutrient-addition bioassays (without time series). By manipulating the light level, we controlled the responses in the bioassay.

This can also be seen in the light responses measured at the end of the incubation (Fig. 22). Chlorophyll a increased in all treatment with increasing light level in the incubations up to

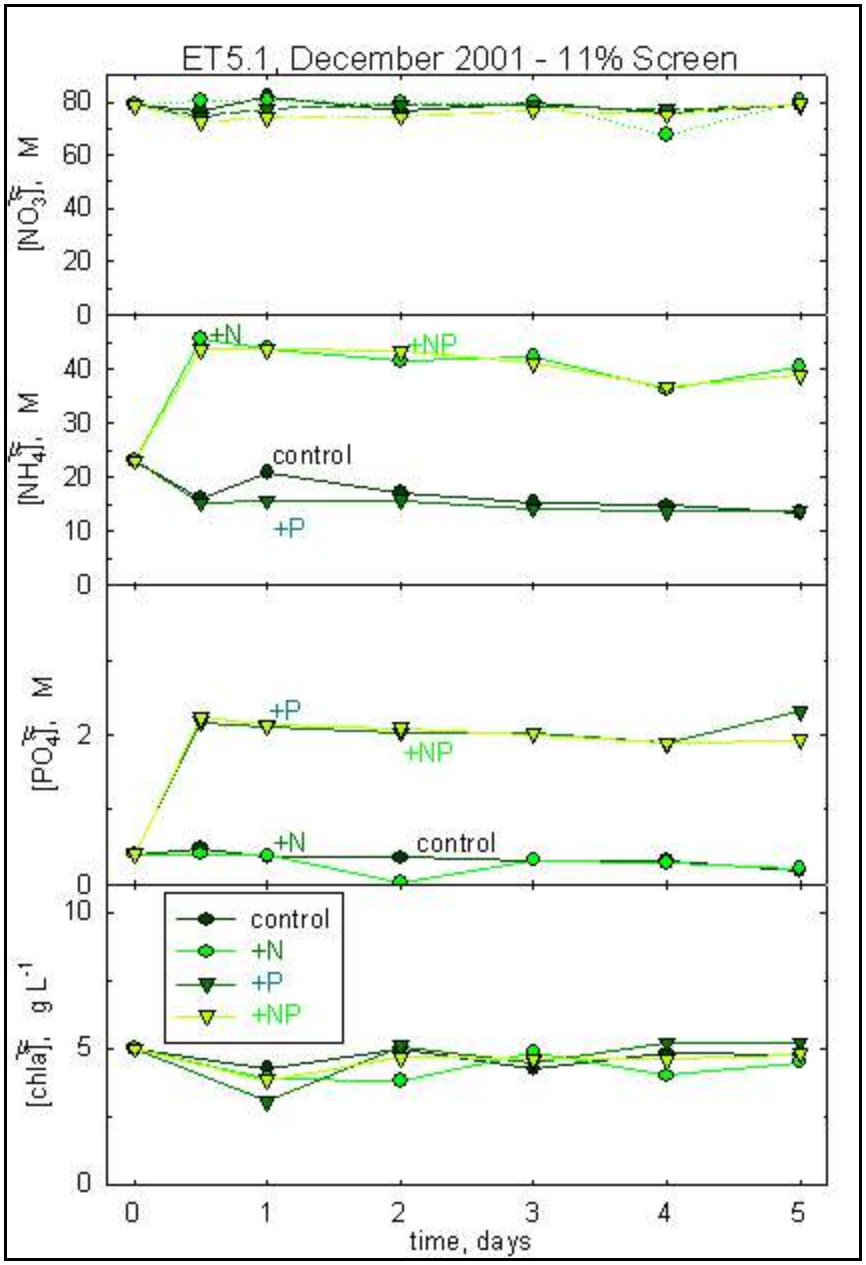

Fig. 20. Resource-addition bioassay at a tidal fresh station in winter. The time series of treatments at 11% light showed essentially no nutrient uptake nor increases in chlorophyll a at this low light level.

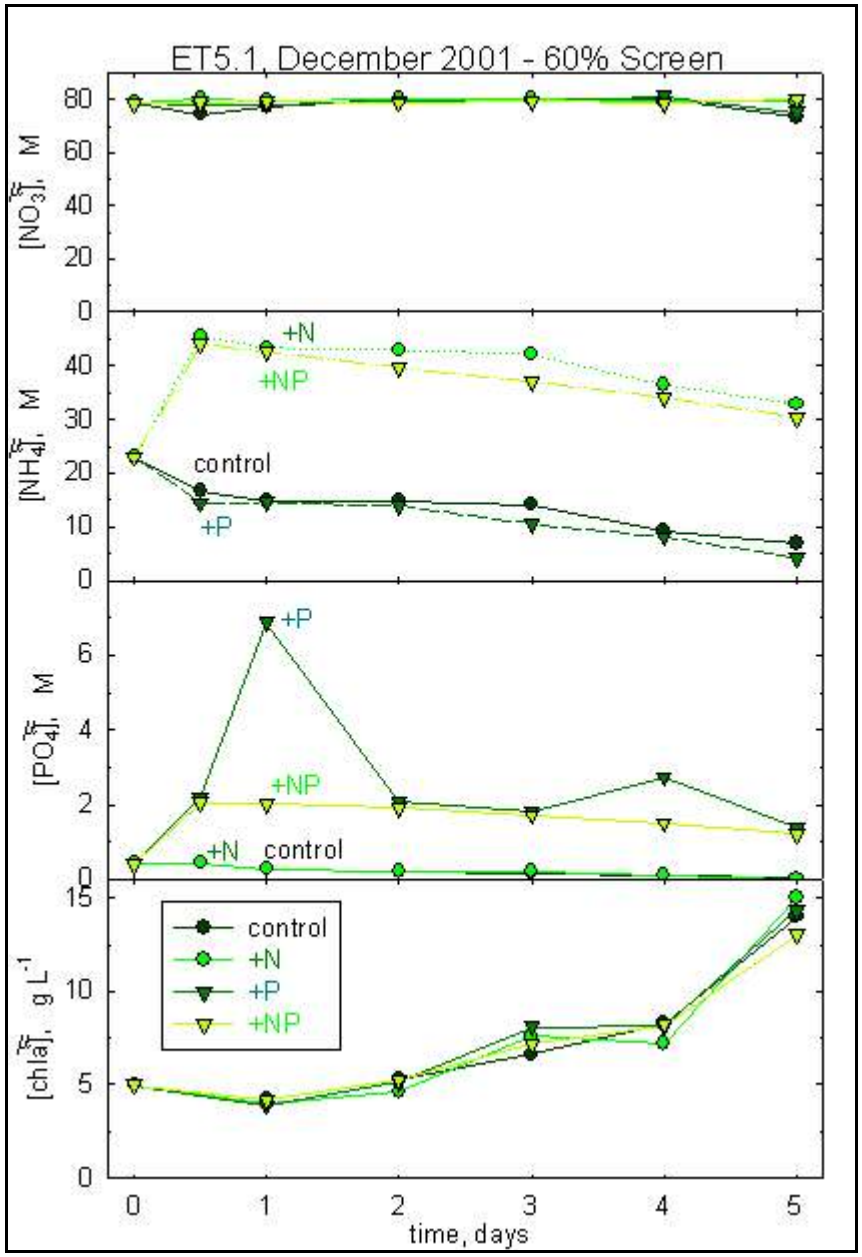

Fig. 21. Resource-addition bioassay at the same time and station as Fig. 1, but at a 60% light level, showing partial uptake of the added nutrients and increases in chlorophyll a in response in all treatments, including the control.

the 60% level, similar to a P vs. I response in a C fixation measurement. However, C fixation appeared to exhibit almost the inverse pattern, with more ^{14}C-CO_2 being fixed at lower light levels than at 60%, again irrespective of treatment. If we normalize the C fixation to chlorophyll a, we can clearly see that more C fixation occurs per unit chlorophyll a at light levels ≤ 20% than at higher light levels (Fig. 23). This clearly shows that adaptation to the lower light levels was occurring, despite the apparent lack of change in chlorophyll a (e.g., Fig. 20). This light adaptation is largely the result of the relatively low and fixed level of light at which we do all of our ^{14}C incubations (200 µE m^{-2} s^{-1}). This is equivalent to ~10% of noon intensity, normally sufficient to saturate photosynthetic C fixation and achieve the maximum rate of photosynthesis (P_m). However, under low light, phytoplankton light-adapt, increasing their photosynthetic efficiency at low light and increasing P_m (shift-up response). Normalized to chlorophyll a (P_m^b), this would appear as in Fig. 23, with increasing response at lower light levels.

A second resource-addition bioassay is summarized in Figs. 24-25. The water sample was collected at mesohaline station LE1.1 in January 2001 and was incubated at the low ambient water temperature (2°C). Initial nutrient and biomass conditions in this bioassay were quite different compared to the bioassay described above: inorganic N was at low levels (<0.5 µM or <0.007 mg N L^{-1}), PO_4 was nearly undetectable (0.1 µM = 0.003 mg PO_4-P L^{-1}), and chlorophyll a was ~13 µg chla L^{-1}. In the time series at the 60 % light treatment (Fig. 24), the added NH_4 and PO_4 were partially consumed, particularly PO_4, but after 5 days there were still persistent and significant residuals of both PO_4 (0.5 µM = 0.015 mg PO_4-P L^{-1}) and NH_4 (28 µM = 0.39 mg PO_4-P L^{-1}). NO_3 remained at low levels (0.2 µM = 0.003 mg NO_3-N L^{-1}), but increased towards the end of the incubation in the +N and +NP treatments, probably due to nitrification of the added NH_4. Despite the relatively high NH_4 and PO_4, chlorophyll a declined during the incubation (lower panel of Fig. 24), and there was no significant differential effect of the added N or P on the accumulation of algal biomass. In the past we would have interpreted this as no response to nutrient additions (NOR) on day 2 or as inconsistent (INC) on day 5, since some treatments were significantly lower than the control. The time series clearly reveals a decline in chlorophyll a relative to the initial, suggesting either light adaptation (changes in chlorophyll a per cell) or control of algal accumulation by grazing, which could exceed growth rates at the low water temperature (2°C) in the presence of excess NH_4 and PO_4. Classifying this bioassay as NOR (light limitation) could be justified had we obtained a positive response (an increase in chlorophyll a) in the light series. However, we did not observe this (see below), and it is likely that light adaption or an excess of grazing over growth rates was responsible for the changes in chlorophyll a shown in Fig. 24.

The light series for the January resource addition bioassay showed inverse relationships between chlorophyll a and ^{14}C uptake with light (Fig. 25). Chlorophyll a on day five in all treatments was 50-100% of the initial value, declining with incubation light level, with a slight stimulatory effect of +N and +NP treatments at light levels <58 %. At the higher light levels (58 and 100%), the effects of the N additions disappeared or were small. For ^{14}C uptake there was a somewhat similar pattern, except that +N and +P were reversed at light levels <58 %, suggesting a P response. At the higher light levels, the effects of the nutrient treatments on ^{14}C uptake disappeared or were small relative to the control. The inverse effect of incubation light level on

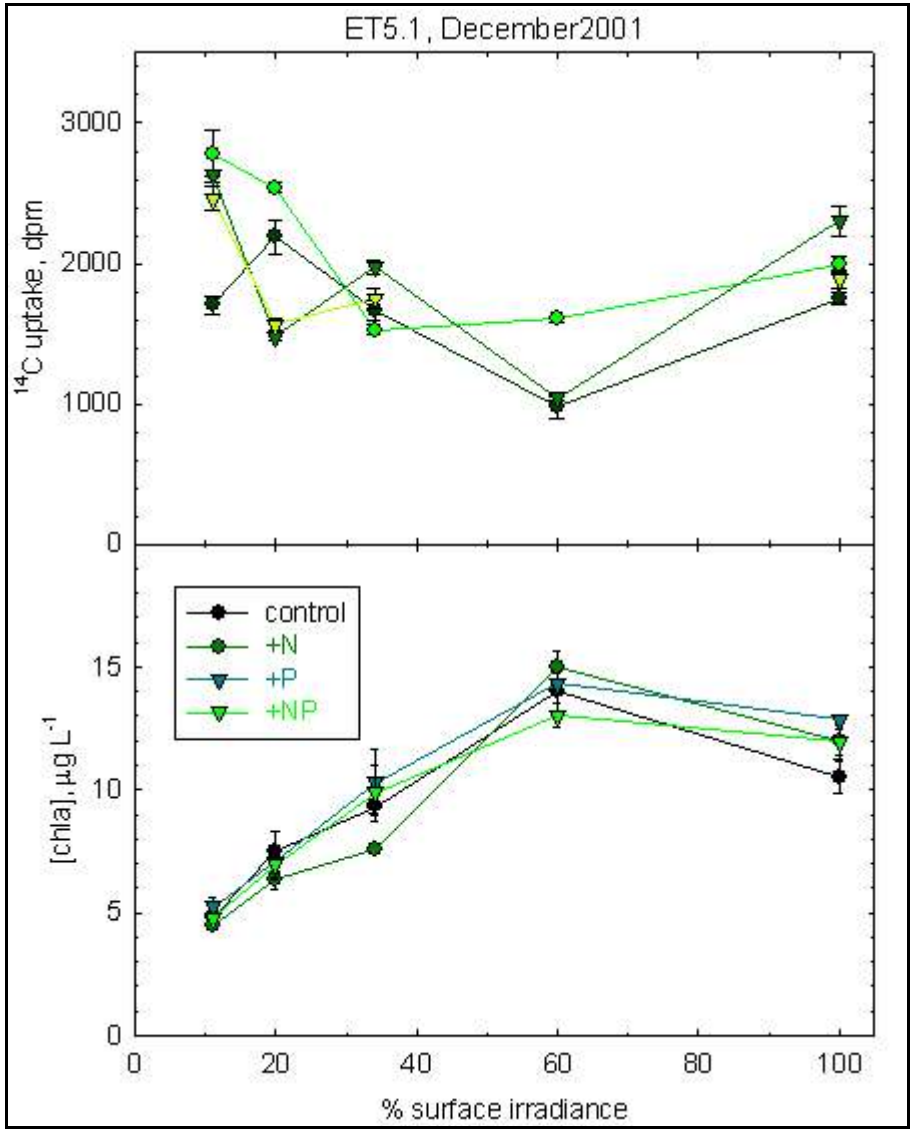

Fig. 22. Final chlorophyll a and C fixation in the resource-addition bioassay shown as a time series at two light levels in Figs. 1-2. After 5 days the amount of chlorophyll a that developed was a function of light level up to 60%. However, C fixation showed an almost inverse pattern.

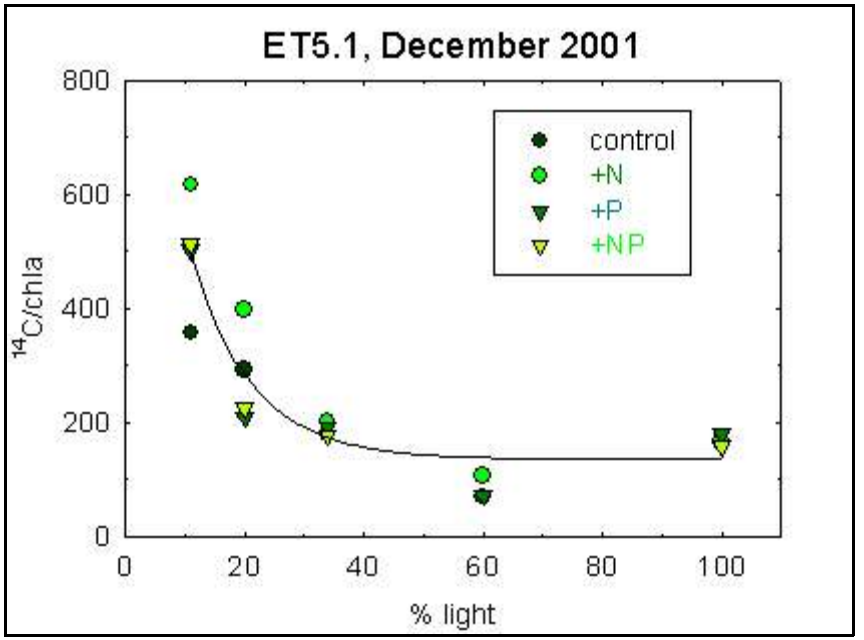

Fig. 23. C fixation per unit chlorophyll a in the treatments shown in Fig. 3. C fixed per unit chla increased at lower light levels, suggesting that adaptation to low light was occurring during the five day incubation. As in Figs. 1-3, there was no differential response to added nutrients, and all treatments responded similarly.

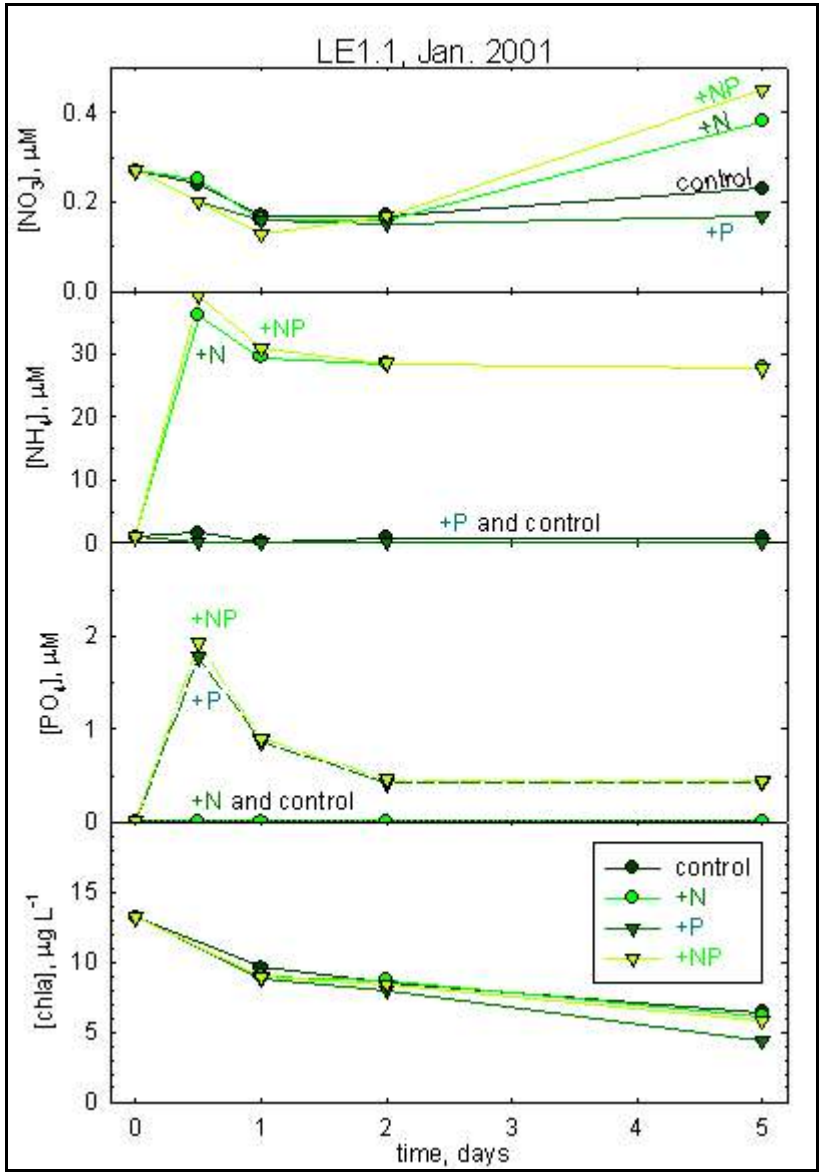

Fig. 24. Time course of nutrients and chlorophyll a in a resource-addition bioassay from a Patuxent sample incubated at the 60% light level.

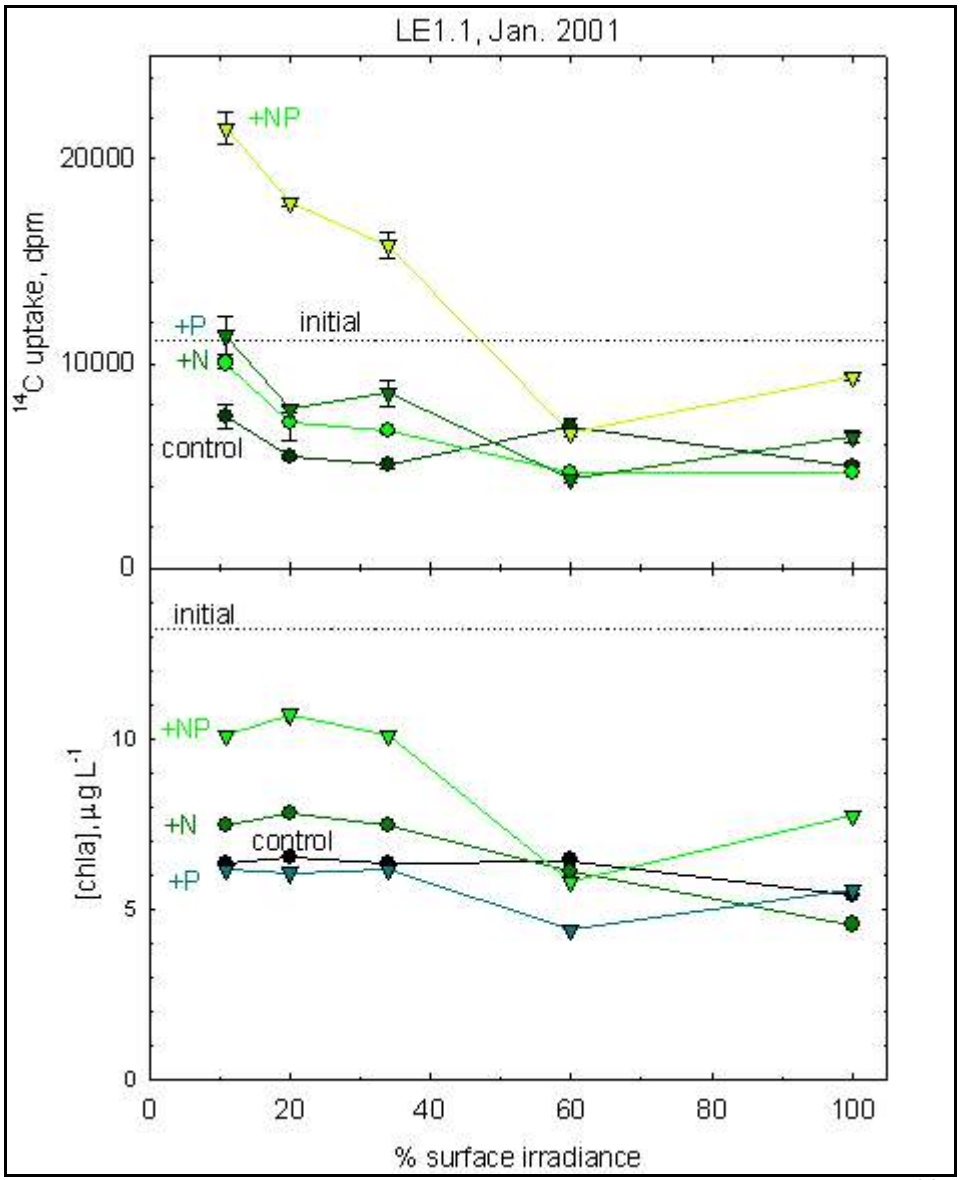

Fig. 25. Light response of chlorophyll a and ^{14}C uptake in the resource-addition bioassay shown in Fig. 5 on day 5 of the incubation.

chlorophyll a and ^{14}C uptake at 200 µE m^{-2} s^{-1} again suggests that light adaptation had occurred during the incubation, and does not support the light limitation interpretation of the time series at 58 % light (no significant increases in chlorophyll a at 100% surface irradiance). None of the chlorophyll a or ^{14}C responses shown in this bioassay were large, contributing to the ambiguity of the interpretation. Depending on light level, we could classify the day 5 responses of chlorophyll a and ^{14}C in Figs. 24-25 as PRN (chla), PRP (^{14}C), or NOR (58 % light).

There was one consistent feature in the resource-addition bioassays. Although details vary between all resource addition bioassays done to date (Table 8), all light series showed inverse relationships between the incubation light level and ^{14}C uptake (e.g., Figs. 22 and 25). In contrast, chlorophyll a showed either a somewhat parallel inverse relationship (e.g., Fig. 25) or a positive hyperbolic relationship (e.g., Fig. 22). Although it is possible that less grazing could occur at lower light levels, the inverse responses to incubation light level shown in Figs. 22 and 25 suggest that light adaption of algal populations has a significant effect on the observed responses in our bioassays. For instance, if an algal population is photoadapted to light levels close to the 58 % light level, then at the lower % light incubations we would expect to see higher chlorophyll a per cell and higher ^{14}C uptake at the relatively low irradiance (200 µE m^{-2} s^{-1}) used in the ^{14}C measurements, as observed in Fig. 23. On the other hand, if the sampled algal population was photoadapted to lower light levels than the 58 % which we normally use in our nutrient-addition bioassays, then at the higher light levels photopigments could be bleached, with detrimental effects on both chlorophyll a and C fixation similar to light inhibition, as observed in Fig. 25. Since nutrient-addition bioassays have been done at the 58 % light level at over 18 stations throughout the bay for 11 years by two groups of investigators (Fisher's and Haas' labs), we consider it important to understand the results in Figs. 23 and 25. The ambiguity in the interpretation of the low temperature bioassay (Fig. 24) may be responsible for the difficulties we have encountered in predicting light limitation in the indicator models, and we plan to continue to explore phytoplankton responses in these resource-addition bioassays in the coming year to broaden our data base and to gain a better understanding of responses at low temperatures, particularly light limitation and the possibility of grazing overcoming growth rates, as we suggested above to explain the results shown in Fig. 24 (excess NH_4 and PO_4 accompanied by declines in chlorophyll a).

Discussion

Nutrient-addition vs. resource-addition bioassays

The resource-addition bioassays provided a mix of expected and unexpected results. For instance, the time series at 58% light in the Dec, 2001 example (Fig. 21) was consistent with previous nutrient-addition bioassays. Nutrients declined, and chlorophyll a increased, similar to our expectations based on previous results at this and other stations (e.g., Fig. 3). Since ~15 µM NH_4 had been consumed by day 2, we can use the average N/chla of phytoplankton biomass (~0.8 µmol N/µg chla, Parsons and Takahashi 1973) to estimate that 18 µg chla L^{-1} should have been produced. With an initial chlorophyll a of ~5 µg chla L^{-1}, we would expect to see ~23 µg chla L^{-1} on day 5, somewhat more than the observed value of ~15 µg chla L^{-1}. Since ambient DIN was not depleted in this resource addition bioassay on day 5, the control and +P treatments showed essentially parallel responses to those of the +N and +NP treatments. The differences between our expected and observed values could be due to grazing or other losses, but the general response pattern fit our expectations.

However, the resource-addition bioassays also provided data that did not fit our expectations. For instance, there was a consistent inverse relationship between incubation irradiance and biomass and ^{14}C uptake in the light series (Figs. 22, 23, 25). We suspect that the decline of ^{14}C uptake with increasing light availability was probably the result of light adaptation of the phytoplankton populations. We had anticipated a positive hyperbolic response between incubation light availability and both chlorophyll a and ^{14}C, similar to what is obtained with P vs. I measurements of primary production, and not unlike the chlorophyll a response in Fig. 22. However, light adaptation of phytoplankton can occur within 5 days, and Fig. 23 provides firm evidence that this occurs in our bioassays. In future resource-addition bioassays, we will make three changes: (1) we will add a second time course in the 6 or 11% light treatment to examine the role of light adaptation within the control and treatments at low light; (2) we will drop the 100% light treatment; and (3) we will add measurements of fluorescence per cell in a flow cytometer to quantify light adaptation.

The ^{14}C uptake data also support the role of shade adaptation in the interpretation of the data of Fig. 22. More C was fixed at 200 µE m^{-2} s^{-1} by treatments incubated for 5 days at low light compared to treatments incubated at higher light levels, suggesting that 200 µE m^{-2} s^{-1} may be closer to the optimal light intensity of the lower light adapted subsamples. Given a maximum irradiance of 2000 µE m^{-2} s^{-1} at noon, the irradiance during the ^{14}C measurements (200 µE m^{-2} s^{-1}) is about 10% of this value. If light adaptation is in fact responsible for the patterns shown in Figs. 22 and 23, it will be necessary to consider the changes in chlorophyll a per cell to interpret the results of incubations at different light levels or to interpret the results when the light level of the bioassay incubation differs from that recently experienced by the sampled population.

Spatial and seasonal variability

We have demonstrated considerable spatial variability in resource limitation in Chesapeake Bay. Tidal fresh stations were turbid and nutrient-rich, and we obtained primarily

light-limited (NOR) bioassays, except in summer when nutrients were occasionally depleted (Figs. 11, 12). During summer, occasional nutrient-depleted conditions resulted in N limitation in the Choptank and Patuxent tidal fresh regions, but P limitation in the tidal fresh Potomac. The mesohaline Patuxent showed brief P limitation only during high river flows in early spring; large sewage inputs with low N/P quickly eliminated P limitation after river discharge with high N/P decreased later in the year (Figs. 8-9); however, the remaining mesohaline stations in the main bay, lower Choptank, and lower Potomac uniformly exhibited a consistent seasonal progression of light, P, and N limitation throughout the year (Fig. 6).

We have related this spatial variation in the bioassay responses to the N/P of inputs reported by Boynton et al. (1995). High N/P of total N and P inputs indicates high availability of N relative to P (tendency to P limitation), and low N/P indicates low availability of N relative to P (tendency to N limitation). Long-term mean indices of resource limitation in mesohaline regions of the MD waters of Chesapeake Bay were significantly related to the N/P ratio of total N and P inputs reported by Boynton et al. (1995), as shown in Fig. 26. As the input N/P increased (increasing N availability), the long-term mean N indices decreased and the long-term mean P indices increased. The index of light limitation also increased significantly with increasing input N/P, probably because the highest nutrient loadings near rivers are associated with high N/P ratios and high turbidity. Note that the Patuxent, with its large sewage inputs, had the lowest N/P, the lowest P index, and the highest N index of all regions. Likewise, the Patapsco (data available only for 1990-1992, see Fisher et al. 1992b) had the highest N/P in inputs, the lowest N index, and the highest P index. Although the input data are from an earlier period (mid 1980's) than the bioassay data (1990's), it is clear that the input ratios are largely responsible for the spatial variability in resource

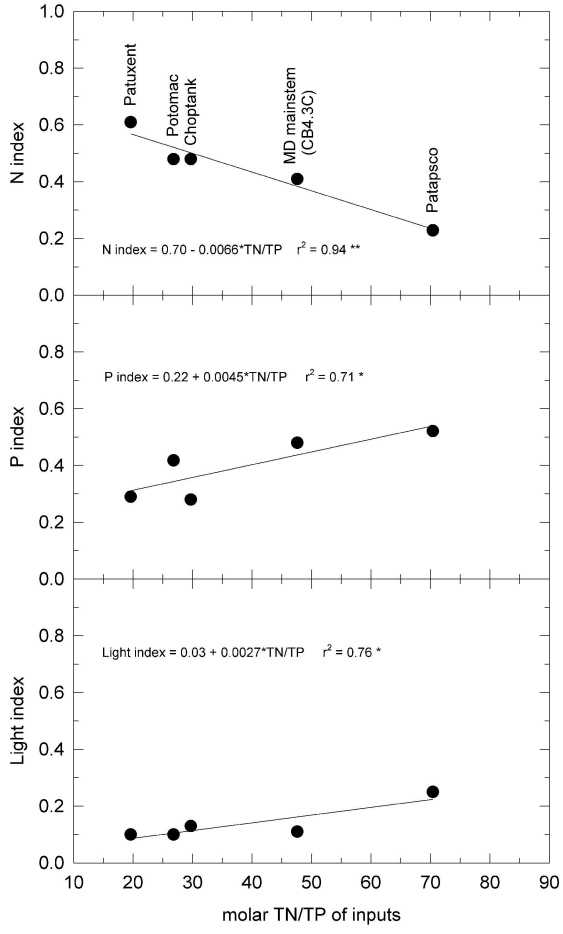

Fig. 26. Long-term mean indices of resource limitation at mesohaline stations (1990-1995) related to the molar ratio of nutrient inputs for the mid 1980's reported by Boynton et al. (1995).

limitation described above and that manipulations of the inputs is likely to be detected by the indices of resource limitation computed from the bioassay results.

River discharge and nutrient content clearly have a strong influence on resource limitation of phytoplankton in Chesapeake Bay. The relationships shown above between the multi-year average indices and N/P input ratios (Fig. 26) illustrate one of, and perhaps the most important, control on spatial variation in resource limitation. Likewise, the relationships between monthly average indices and river flow (Figs. 7, 10, 13 and Table 6), show one of the most important controls on seasonal variation in resource limitation. However, other processes also influence seasonal variations in resource limitation (e.g., the onset of seasonal anoxia, and fall overturn of the water column).

Status and trends

The data presented above have been used to indicate the status of resource limitation at the nine stations. The initial status of these parameters was computed over the first two years of the project (August, 1990 to July, 1992), and the current status was computed over the last three years of the project (January, 1998 to December, 2000). Data from months in which double sampling occurred (May, June, July) were averaged to produce monthly averages to avoid bias due to the large seasonal variations shown in Figs. 6, 9, and 12. Missing data were interpolated between months to avoid biasing an annual average; and, at tidal fresh stations, data were compiled only over the months of May, July, and September to correspond with our current sampling periods.

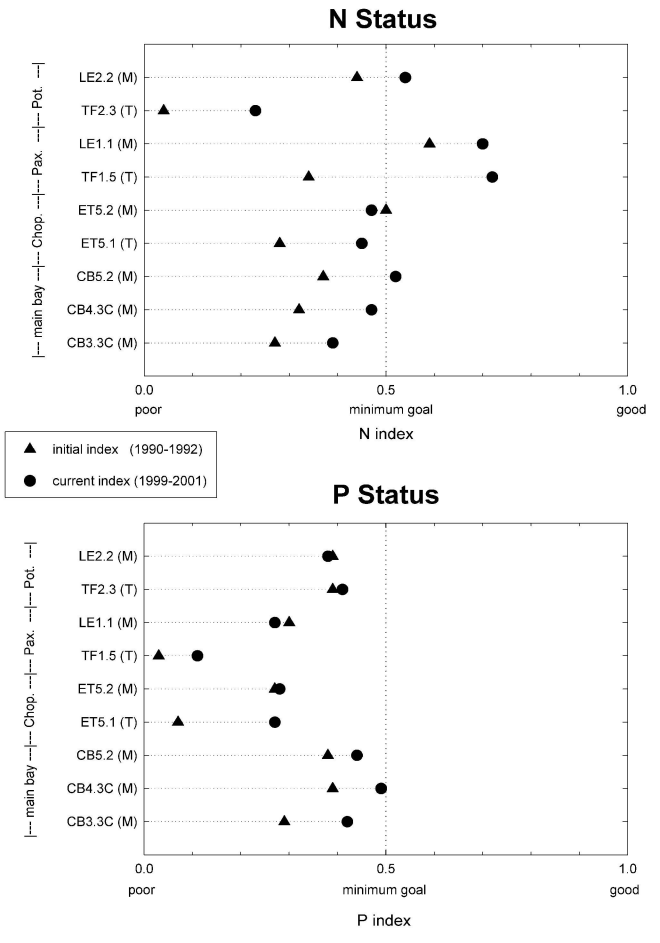

Fig. 27. Status of N and P indices of nutrient limitation of phytoplankton at 9 stations in MD waters of Chesapeake Bay. Station types (M=mesohaline, T=tidal fresh) are indicated in parentheses. Note: Station CB3.3C current index is from 1998-2000.

The status of nutrient limitation generally showed improving water quality over the decade (Fig. 27). Eight of the nine stations exhibited greater N limitation in the

last three years (1999-2001) compared with the initial period (1990-1992). Only the Choptank tidal fresh station showed a decline in N limitation due to increasing P limitation at this station (Table 6). The P status was similar, but somewhat more mixed. Five of the nine stations showed improvement, and four stations (tidal fresh and mesohaline Potomac, mesohaline Patuxent and Choptank) showed little or no change. The increases in the indices at most stations indicate increasing N and P limitation and less light limitation or nutrient saturation between the two time periods, whereas the lack of change in the indices at the Potomac, Patuxent, and Choptank mesohaline stations indicates no improvement in water quality. For both indices, the minimum goal for each index has been set at 0.5. Since the indices sum to 1.0, each station can only achieve a total of 1.0 between the two indices, assuming control of growth rates by either N or P and no light limitation. Both Patuxent stations exceeded the minimum goal for N (LE1.1 and TF1.5), as did the mid-Bay station CB5.2. Only mid-Bay station CB4.3 approached the minimum goal for P. Only the mid-Bay mesohaline stations CB4.3C and CB5.2approached the minimum goal for both nutrients (N+P index = 0.9), and the remainder of the stations indicated some nutrient saturation and light limitation during the year. These data suggest that water quality in the MD portion of the Bay has improved somewhat in the 1990's, but that continued reductions in nutrient inputs from the surrounding watersheds are still required in order to achieve the water quality goals of the Bay Program.

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